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Modeling lichen communities: ecological key factors in a changing environment

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Resumo

O fenómeno das alterações globais influencia o funcionamento de muitos dos sistemas planetários. Embora os factores ambientais associados a esse fenómeno funcionem numa escala global, os seus efeitos nos ecossistemas têm de ser estudados localmente. Este estudo é complexo não só pela necessidade de obter informação com uma elevada resolução espacial, mas também pela dificuldade de estarmos a trabalhar com diferentes escalas espaciais de análise e potenciais interacções entre os factores ambientais. Embora as medidas fisico-químicas tenham de ser usadas para determinar as variações dos factores ambientais, não é possível ter um número de estações adequado, de modo a obter uma monitorização espacial de possíveis alterações ambientais, especialmente quando o momento ou mesmo a presença de um determinado factor ambiental não são conhecidas. Para além disto, as medidas fisico-químicas não podem mostrar directamente que uma alteração ambiental causou um efeito biológico. A utilização de indicadores ecológicos pode ajudar a eliminar estas limitações, ajudando na identificação das áreas críticas sobre a influência dos factores associados às alterações globais, de modo a diminuir o seu impacto e determinar as prioridades para acções de gestão.

O objectivo geral desta tese é fornecer um enquadramento geral para a utilização da diversidade de líquenes como indicadores ecológicos integradores de mudanças ambientais em ecossistemas Mediterrânicos. Isto foi feito através da análise das respostas da comunidade liquénica a factores ambientais, de modo a seleccionar variáveis-liquénicas como potenciais indicadores ecológicos. Esses indicadores foram depois modelados contra os factores ambientais chave, incluindo os associados às alterações globais (eutrofização, clima e poluição atmosférica). Para isso foi utilizada uma análise espacialmente explícita, de forma a desagregar/discriminar as respostas das comunidades liquénicas a múltiplos factores ambientais. Por último os indicadores ecológicos foram aplicados na monitorização dos ecossistemas mediterrânicos num ambiente em mudança.

Os factores ambientais funcionam a diferentes escalas espaciais, estando muitas vezes sobrepostos em algumas áreas. De foram a utilizar líquenes como indicadores ecológicos da influência simultânea de múltiplos factores ambientais, é necessária uma análise espacial explícita, o que foi feito no **sub-capítulo 2.1**. Deste modo, foi demonstrado que os líquenes respondem a factores ambientais que funcionam a diferentes escalas espaciais, e que cada factor teve uma distância de influência própria, o que se deveu aos diferentes padrões de dispersão de poluentes. Neste capítulo foi salientada a importância de uma análise espacial explícita para interpretar as relações entre as alterações das variáveis-liquénicas e os factores ambientais associados.

Para além de uma análise espacial, as variáveis-liquénicas baseadas na diversidade funcional são ferramentas promissoras como indicadores ecológicos, especialmente se puderem ser usadas para desagregar a influência de múltiplos factores ambientais. A resposta das espécies de líquenes a factores ambientais chave foi estudada no **sub-capítulo 2.2** onde se demonstrou que essa resposta dependeu das características funcionais das espécies, permitindo a definição de grupos de espécies em dois grupos funcionais. Este capítulo mostrou que os dois grupos-funcionais de líquenes podem ser o elo entre uma resposta ecofisiológica conhecida e os factores ambientais que a provocam, destacando as enormes vantagens ao considerar grupos-funcionais como indicadores ecológicos, em comparação com a riqueza específica.

No **capítulo 3**, dois grupos funcionais de líquenes foram estudados num gradiente espacial de alterações micro-climáticas. Um mapa mostrando os efeitos das alterações microclimáticas pôde assim ser feito com uma elevada resolução espacial. O período chave em que as alterações micro-

climáticas têm maior efeito nas comunidades liquénicas foi abordado, tendo em consideração as características associadas a cada um dos grupos funcionais.

Uma vez que a maioria dos ecossistemas Mediterrânicos não são naturais, mas sim áreas semi-naturais com baixa intensidade de uso do solo, e há um profundo desconhecimento deste tipo de impacte, foi importante determinar qual a influência desta baixa intensidade de uso do solo nos líquenes. Isto foi feito no **capítulo 4**, onde a influência de uma baixa intensidade do uso do solo nos líquenes foi estudada num montado tradicionalmente gerido. Os resultados mostraram que os dois grupos funcionais responderam ao gradiente de intensidade de uso do solo, com as espécies sensíveis à eutrofização a diminuírem em abundância, enquanto as espécies tolerantes à eutrofização aumentaram. É importante salientar que a intensidade de uso do solo foi suficientemente baixa para que as espécies sensíveis à eutrofização não diminuíssem em riqueza, mostrando a capacidade da gestão tradicional do montado em manter um alto nível de riqueza de espécies.

Um dos factores ambientais que influenciaram os líquenes em todos os capítulos anteriores foi a actividade agrícola. A maioria dos efeitos das actividades agrícolas na biodiversidade dos ecossistemas terrestres é devida aos efeitos da amónia atmosférica (NH_3), embora os efeitos isolados deste poluente atmosférico nos líquenes não seja bem conhecido, especialmente em ecossistemas Mediterrânicos. Isso foi estudado no **capítulo 5**, num montado sob a influência de uma fonte isolada de NH_3 (uma vacaria). A maioria das alterações nas espécies de líquenes e grupos funcionais foram explicadas pelas concentrações de NH_3 atmosférica, medida directamente por deposímetros físicos. Desta forma foi possível mapear com um elevado detalhe as áreas de diferente impacte da NH_3 atmosférica no montado. A classificação das espécies em grupos funcionais, baseada no conhecimento de peritos, pôde ser pela primeira vez testada em ecossistemas mediterrânicos num gradiente de concentrações de NH_3 atmosférica.

Utilizando a relação obtida no capítulo anterior, onde a NH_3 atmosférica explicou a maioria das alterações das comunidades liquénicas (cap.05) foi possível calcular o nível-crítico de NH_3 atmosférica, o que foi também conseguido pela primeira vez em ecossistemas Mediterrânicos. Neste estudo foram testadas diversas variáveis-liquénicas, incluindo a diversidade total e funcional, no **capítulo 6**. Os níveis críticos encontrados variaram entre 1 e 2 $\mu g\ m^{-3}$, o que contribuiu para a proposta de revisão dos níveis críticos na Europa, anteriormente fixados em 8 $\mu g\ m^{-3}$.

Uma vez que, num contexto de múltiplos factores ambientais, existem factores que potencialmente podem causar a mesma resposta nos líquenes, uma outra estratégia para a utilização de líquenes como indicadores ecológicos foi utilizada. Deste modo, no **capítulo 7** foi possível relacionar o azoto total medido nos líquenes com as emissões de NH_3 atmosférica estimadas de duas formas diferentes a uma escala regional. Os resultados mostraram que as concentrações de azoto nos talos liquénicos estavam significativamente relacionadas com as emissões de NH_3 atmosférica estimadas. Para além disto foram utilizadas as ferramentas de análise espacial desenvolvidas anteriormente para mapear as áreas do território em risco devido ao potencial impacto da NH_3 atmosférica, em especial dentro da rede Natura 2000.

Finalmente, o objectivo no **capítulo 8** foi o de aplicar o conhecimento obtido nos capítulos anteriores, numa região muito mais complexa e heterogénea, com diferentes tipos de uso do solo e múltiplas fontes de poluição. Utilizando uma análise espacial explícita, foi possível individualizar as áreas de impacte de vários factores ambientais e dos poluentes associados, possibilitando assim a determinação dee uma escala espacial para cada um dos factores estudados. Isto permitiu desagregar os efeitos de múltiplos factores ambientais e apoiar o uso de líquenes como indicadores de alterações ambientais num ambiente complexo e com múltiplas fontes de contaminação.

No **capítulo 9** foi apresentada uma discussão geral, integrando os resultados dos capítulos anteriores, em especial no que se refere à utilização de líquenes como indicadores ecológicos e dos efeitos associados aos factores das alterações globais num contexto Mediterrânico. Ao longo deste trabalho foi demonstrado que os líquenes responderam a um elevado número de factores ambientais, e a uma escala larga de intensidades. Por tudo isto, foi possível reafirmar o valor dos líquenes como indicadores ecológicos e demonstrar o seu valor único como integradores universais de largo espectro das alterações ambientais. A utilização de medidas de abundância como o LDV (Lichen Diversity Value) foi preferível às medidas de riqueza específica, sempre que os factores ambientais eram de baixa intensidade. Um resultado fundamental deste trabalho foi a demonstração de que a diversidade funcional é uma medida complementar às medidas de diversidade total, porque espécies diferentes podem ter respostas opostas ao mesmo factor ambiental, tal como foi mostrado para a amónia atmosférica. Algumas estratégias inovadoras foram utilizadas para desagregar o efeito de múltiplos factores ambientais nos líquenes, permitindo realçar os efeitos dos factores ambientais que pretendíamos estudar. Uma dessas estratégias envolveu a manutenção de efeito constante dos factores ambientais sem interesse para o estudo, fazendo com que o seu efeito nos líquenes fosse homogéneo em toda a área estudada. Outra estratégia foi a utilização da diversidade funcional, que mostrou ser um indicador ecológico robusto e preciso. Para uma melhor identificação da origem das causas das alterações das variáveis-liquénicas, o uso do solo na vizinhança dos locais de amostragem foi também utilizado. Finalmente, através da medição da concentração de poluentes acumulados nos líquenes foi possível localizar as fontes poluidoras.

Uma análise espacial explícita mostrou que os líquenes são influenciados por múltiplos factores que funcionam no mesmo território a diferentes escalas espaciais, o que foi relacionado com o tamanho das partículas/poluentes que são dispersos por cada tipo de uso do solo. Desta forma foi possível interpretar mais correctamente relação entre as alterações das variáveis-liquénicas e os factores ambienteis associados.

Tendo em conta os resultados obtidos, foi possível mapear com confiança estatística, as áreas em risco devido à eutrofização, poluição atmosférica e alterações micro-climáticas. Outras aplicações poderão incluir o uso de líquenes como indicadores precoces de transições críticas nos ecossistemas, bem como a utilização de líquenes como indicadores universais das alterações ambientais.

Palavras chave: alterações globais; indicadores-ecológicos; diversidade funcional; grupos-funcionais ; análise espacial;

Abstract

The ongoing global change affects many planetary systems functioning. Although the environmental factors associated to global change work at a planetary scale, their effects on ecosystems must be assessed locally. This assessment is complicated by the need to have information with high spatial resolution and taking into account the different spatial scales of analysis and potential co-occurrence of environmental factors. Although physical-chemical monitoring stations must be used to measure variations in many environmental variables, they cannot be located everywhere and cannot measure all possible environmental changes, especially when the time of change or even the presence of some of environmental factor is not known. Additionally, physical-chemical monitoring stations cannot provide any evidence that the environmental change caused a biological impact. The use of ecological indicators could fulfil these gaps, helping in the identification of the critical areas under the influence of the factors associated to global change in order to ameliorate the impacts or rank priorities.

The general aim of this thesis was to provide a general framework for the use of lichen functional-diversity as an integrating ecological-indicator of environmental changes in Mediterranean type ecosystems. This was supported by the analyses of the lichen community responses to environmental factors in order to select of lichen-variables as potential ecological indicators. The selected indicators could then be modelled against the key environmental factors, such as the ones associated to drivers of global change (eutrophication, climate and, air pollution). For that, a spatial explicit analysis was used in order to disentangle the response of lichen communities to multiple environmental factors and ultimately to apply the selected ecological indicators to monitor the complex Mediterranean-type ecosystems in a changing environment.

Environmental factors work at different spatial scales, sometimes superimposed in some areas. To use lichens as ecological indicators of the influence of multiple environmental factors, a spatial explicit analysis was necessary, which was done in **sub-chapter 2.1**. Lichens were shown to respond to environmental factors working at different spatial scales and each factor had a particular distance of influence, which was due to the different patterns of pollutants dispersion. This chapter highlighted the importance of a spatial explicit analysis to interpret the relation between lichen-variables and the underling environmental factors.

Besides a spatial analysis, lichen variables based on functional-diversity are promising tools as ecological indicators, especially if they can be used to disentangle the influence of multiple environmental factors. The response of lichen species to the key environmental factors was studied in **sub-chapter 2.2** and was shown to be dependent on their functional characteristics, which allowed to group species into functional groups. This chapter showed that lichen functional groups can provide a link between a known physiological response and the environment factor to monitor, highlighting the advantages of considering functional groups as ecological indicators.

To know the spatial and temporal patterns of response of lichen-variables to the drivers of global change we used a spatial explicit analysis (ch.02.1) and functional groups (ch02.2). In **chapter 3** two lichen functional groups were studied in a spatial gradient of climate alterations. A high resolution mapping of the effect of climate alterations was obtained. The key period of the year that has the greatest effect on lichen functional groups was discussed within the framework of the particular physiological traits of concern.

Because most Mediterranean ecosystems are not natural but rather semi-natural areas with low intensity land-use, it was important to determine the influence of low-intensity land-use on lichens,

which is rarely studied. This was done in **chapter 4**, where the influence of low-intensity land-use in lichens was assessed in traditionally managed Mediterranean woodlands. The results have shown that lichens functional groups responded to low-intensity land-use, with eutrophication sensitive species decreasing in abundance while tolerant ones increased. Interestingly, the level of land-use intensity was such that the number of sensitive species has not decreased, revealing the capability of traditional Mediterranean management to support high species richness. These results highlighted the possibility of using lichens as ecological indicators of low-intensity environmental changes and thus as early-warning indicators for the first effects of those changes.

One of the key environmental factor influencing lichens in all previous chapters was associated to agriculture activities. Most effects of agriculture on terrestrial biodiversity are due to atmospheric ammonia (NH_3) although the isolated effect of this air pollutant on lichens species and functional groups is not well understood, especially for Mediterranean areas. This was studied in **chapter 5**, in cork-oak woodland influenced by a single point source of NH_3. We showed that most changes that occurred in species distribution and on functional groups could be explained by the measured atmospheric NH_3. This allowed mapping with high confidence the area of impact of NH_3 in woodland.. The expert-knowledge classification of lichen species into functional groups could be tested explicitly for the first time under field conditions with an isolated source of atmospheric NH_3.

Using the relation obtained in the previous chapter, where NH_3 explained most variance of lichens functional groups (ch05), it was possible to calculate the critical level of NH_3 for Mediterranean woodlands. This was done testing several variables based on lichens, including total and functional diversity, in **chapter 6**. The critical levels were found to be between 1 and 2 $\mu g\ m^{-3}$, what contributed for the proposal for revision of the current critical levels in Europe that were previously set on 8 $\mu g\ m^{-3}$. For the first time it was possible to determine the critical level of atmospheric NH_3 in Mediterranean woodlands.

Because in a multiple factor context potential confounding factors might cause the same response in lichen community, an additional strategy of using lichens as ecological indicators was employed. This was done in **chapter 7** by relating total nitrogen measured in lichens to atmospheric NH3 emissions, estimated in two different ways at the regional scale. The results have shown that nitrogen concentrations in lichens were significantly correlated with NH3 estimated emissions. Additionally we used the spatial analysis tools developed on the previous chapters to map the areas at greatest risk, especially within Natura 2000 network.

Finally, in **chapter 8** the goal was to apply the developed framework for using lichens as ecological indicators, on a large, complex region with different land-uses and multiple sources of air pollution. The simultaneous influence of natural and anthropogenic factors, working at different spatial scales in a large and patchy Mediterranean region, was studied using lichen functional groups as ecological indicators. Using a spatial explicit analysis, the areas of impact of the key environmental factors and associated pollutants were determined, and the spatial scale associated to each environmental factor could also be determined. This allowed to disentangle the impact of multiple environmental factors and to support the use of lichens as ecological indicators in a complex landscape in a changing environment.

In **chapter 9** a general discussion was presented, integrating the key results from the previous chapters, concerning the use of lichen diversity as an integrating ecological indicator for the effect of global change factors in Mediterranean-type ecosystems. During this work it was shown that lichens responded simultaneously to a large number of environmental factors, including to the factors associated to global change, and on the a wide range of factor intensities. Thus, lichens could be considered integrative and wide-range ecological indicators. The use of abundance measures, such as

LDV (Lichen Diversity Value) was found to be preferable to measures of total richness when the environmental factor is of low intensity. A central outcome of this work was that functional diversity was complementary to the measures of total diversity, because different species can have oposing responses to the same environmental factor, as was shown for atmospheric NH_3. A number of innovative strategies were used to disentangle the effect of multiple factors in lichens, allowing us to focus on the effects of the environmental factors of interests. The first, at the sampling design phase, was holding constant the intensity of unwanted factors in order to make their effect on lichens homogeneous within each study. The second was the use of lichen functional diversity that was shown to be an accurate and robust ecological indicator. To further identify the origin and causes of changes in lichen-variables, the land-cover in neighbourhood was used. This analysis was further improved by measuring the amount of elements or pollutants accumulated in lichens. A spatial explicit analysis showed that lichens were influenced by environmental factors working in the same territory but at different spatial scales, which could be related to the size of pollutants/particles predominantly dispersed by each land-cover type. This highlighted the importance of a multiple spatial scale of analysis to deeply interpret the relation between lichen diversity and the underling environmental factors.

By taking into account the work develop we could map lichen-variables with high statistical confidence, for locating the areas at risk due to eutrophication, air pollution and microclimate alterations. Further applications of lichens could include their use as early-warning indicators for critical thresholds in ecosystems. We expect that lichen functional groups, within the framework developed on this work could also be used as universal ecological indicators of environmental changes.

Key-words: global change; ecological-indicators; functional diversity; lichen functional-groups; spatial-analysis;

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Table of Contends

Chapter 1

01 | General Introduction

GLOBAL CHANGE, LOCAL EFFECTS

As a result of human activities, terrestrial and marine ecosystems are experiencing changes at a global scale with global to local effects on ecosystems (MEA, 2005a). Steffen and co-authors (Steffen et al., 2004) point out several indirect drivers for these changes, which are related to increasing human population: demands for food, recreation, mobility, water, safety and comfort. These changes, observed and denounced in the middle of last century (Carson, 1962), are currently being studied in a planetary framework, which considers earth as a system, with global to local functions and fluxes (Fig. 1). Within this framework all changes affecting the Earth systems are known as global change (Steffen et al., 2004). Global change can significantly modify the structure and functioning of ecosystems, and therefore substantial resources have been invested in understanding its effects (IGBP, 2007). The effects of these changes are now considered so large that a new era was proposed, the "Anthropocene" (Crutzen, 2002; Zalasiewicz et al., 2010) It is important to note that global change is far more than climate change (IGBP, 2007). Among the documented changes we can find alterations in the composition of the atmosphere (including air pollution), changes in global and regional climate, habitat destruction and land-cover changes, increase in species extinction rates, increase in the number and effect of exotic species and increase in nitrogen fixation among others (IGBP, 2007; Steffen et al., 2004). The requirement to know the effects on ecosystems of these factors associated to global change is therefore a major need, not only to maintain biodiversity but also to sustain the possibility of ecosystems to provide all services needed for humanity (MEA, 2005b).

Global change and its main drivers

To integrate the global change phenomena in a common approach, recently, Rockstrom and colleagues provided a conceptual framework with nine planetary systems (Rockstrom et al., 2009b) or drivers associated to global change. For each of these planetary systems a boundary was established, representing a safe operation space for humankind (Fig. 1). These authors considered that for three of the systems the boundaries have already been crossed due to human activities: rate of climate change, rate of biodiversity loss and rate of interference with the nitrogen cycle (Rockstrom et al., 2009b). If changes continue to occur at current rate, the systems may reach a tipping point or threshold, beyond which rapid changes may occur (Scheffer et al., 2009). Besides these three systems, the authors were not able to quantify the boundaries for two other planetary systems: atmospheric aerosols loading and chemical pollution. It is important to note that these systems influence each other (Brook et al., 2008; Rockstrom et al., 2009b). For example, it was considered that among the main direct drivers of biodiversity loss and changes in ecosystems function, climate change and eutrophication were the ones that will have an increasing effect on all earth biomes in the future (MEA, 2005a).

Although the influence of global change in planetary systems can be studied within a planetary framework, the effect on ecosystems is the result of the cumulative effects of global to local environmental factors or drivers (Russell et al., 2009). One example of this is the cumulative effect of ocean acidification (global factor) and local pollution (local factor) on subtidal rocky habitats communities. Knowing the effects of these factors at local scales can empower management both at local and global scale. Changes in the factors associated to global change are mostly measured in a planetary assessed variable, such as CO_2 concentration. However, a universal and comparable assessment of local effects between countries or ecosystems is nearly absent. Exceptions are all measures made by remote sensing methods, for example the ones that allow following, at a

planetary scale, the forest cover loss (Hansen et al., 2010). However, the same authors point out that although they are able to characterize the changes, local assessment of the effects is needed (Hansen et al., 2010). Thus, knowing the effects of global change factors at a local scale is severely hampered by the lack of spatial resolution and ground thuthing.

Another major missing approach in characterizing and measuring the effects of global change drivers in ecosystems is the absence of tools aiming at measuring the first changes, which are early-warnings of degradation of ecosystem functioning. If the establishment of early-warning indicators was possible, one could act appropriately to prevent or ameliorate the effect of global change drivers. A further reason for detecting the first effects of global change drivers and implement preventive environmental management, is that the response of ecosystems is not necessarily linear (Steffen et al., 2004). An example is the modification that occurred in the Sahara region, with the abrupt onset and termination of a humid period (deMenocal et al., 2000).

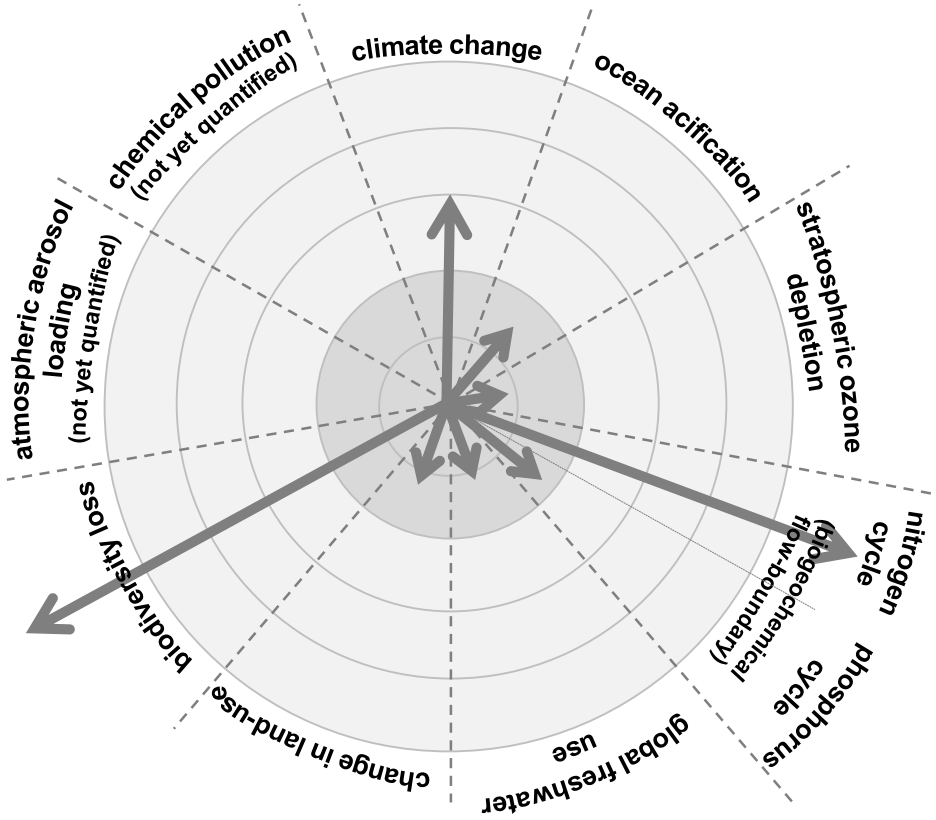

Figure 1: Planetary boundaries system proposed by Rockström and colleges (Rockstrom et al., 2009b). The inner circular shade is the proposed space that can be considered as safe for human activities. The arrows sign the current position for each planetary system. According to this research at least three of the systems may have already passed the safe boundaries: rate of climate change, rate of biodiversity loss and rate of interference with the nitrogen cycle.

Changes in Mediterranean-type ecosystems: a general perspective

The global species richness per area pattern follows a well-known latitudinal gradient, increasing from the poles to the tropics. Among the factors proposed to explain this gradient for plants we can find the influence of the contemporary and past climate, the sensitivity to frost or drought and also geological and topographical background of each region (Kreft and Jetz, 2007). Another group of hypothesis highlights the importance of potential

evapotranspiration, number of wet days per year and topography and habitat heterogeneity (Farina et al., 2005). In fact, in the Mediterranean basin area we can find a large variety of land-cover types in relatively small areas,(Blondel and Aronson, 1999; Farina et al., 2005), where small areas with high conservation value are mixed with low-intensity agriculture and small urban settlements. Moreover, Mediterranean-type ecosystems, together with tropical forests, constitute an important fraction of the biodiversity hotspots (Fig. 2) originally proposed by Myers and colleagues (Mittermeier et al., 2005; Myers et al., 2000). This status is given by the high amount of endemic species and the loss of its original cover.

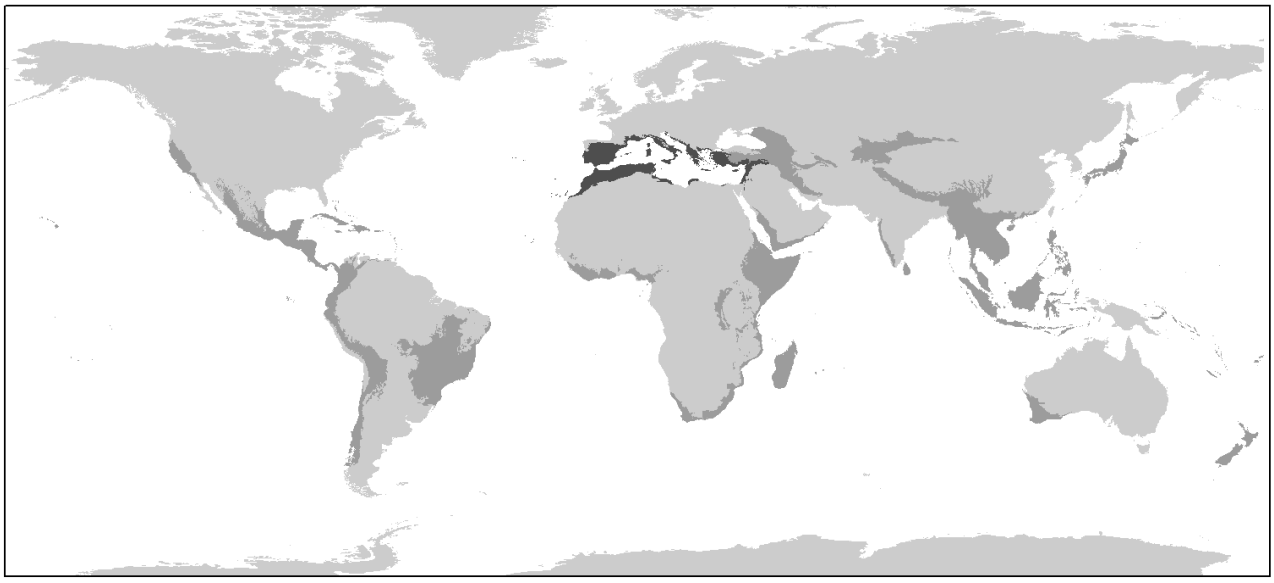

Figure 2: World Biodiversity hotspots, adapted from (Mittermeier et al., 2005). The "Mediterranean-basin" hotspot area is highlighted. This classification includes the areas that have a large proportion of endemic plant species, and, at the same time have lost most of its original vegetation area.

Mediterranean-type forests, which occupy *c.* 7.5% of the Mediterranean basin land area, are diversity-rich ecosystems (Palahi et al., 2008), but, with the exception of those in North America, are among the most fragmented forests in the word (Wade et al., 2003). These ecosystems are part of a mosaic-type landscape where areas of low-intensity human activities surround several Mediterranean forests with high conservation value. At the patch level the effects of forest fragmentation are mostly associated to changes in forest microclimate, generally assumed to be the main component of the so called edge-effects (Chen et al., 1999; Murcia, 1995). Microclimate changes associated to forest fragmentation include increased wind velocity, higher temperatures and light availability, and higher vapour pressure deficit at edges (Laurance, 2008; Laurance and Curran, 2008; Murcia, 1995). Microclimate changes are considered one of the main drivers for fragmentation-associated effects, such as changes in: species abundance, community composition and in ecosystem functioning (Belinchon et al., 2007; Ewers and Didham, 2006; Laurance and Curran, 2008; Ries et al., 2004). Moreover, it has been seen that pollutants, especially pesticides from nearby matrix areas, may penetrate within the forest (Gove et al., 2007), and that access to forest by humans or cattle and invasion by alien species is enhance in agriculture edges (Broadbent et al., 2008; Hamberg et al., 2008; Hobbs, 2001; Kupfer et al., 2006). However, most of these studies were performed in tropical or temperate forests and the underlying factors associated to the fragmentation of Mediterranean forest remain poorly studied. The few studies in Mediterranean forests concerning fragmentation revealed its influence on plant and lichen diversity (Aparicio et al., 2008; Belinchon et al., 2007; Gonzalez et al., 2009).

Another characteristic of Mediterranean landscapes is the low-intensity land-use. In Mediterranean areas

we can find a large area occupied by low-intensity land-use, characterized by a low nutrient input and relatively low output per area (Plieninger et al., 2006). These traditionally land-use managed systems that are species rich are nowadays the target of conservation policies (Plieninger et al., 2006). Many of these areas were classified as "High Nature Value Farmland" (Paracchini et al., 2008). The percentage of total farmland area that could be classified as High Nature Value Farmland reaches 32% overall in Europe, although a difference exists between Mediterranean (52%) and non-Mediterranean countries (27%) (Paracchini et al., 2008). The effect of land-use in species and communities is not straightforward to evaluate. Areas with more intensive forestry activities are usually less species rich (Boudreault et al., 2000; Dettki and Esseen, 1998; Humphrey et al., 2002; Newmaster and Bell, 2002) and have a lower plant abundance or biomass (Dettki and Esseen, 1998; Lehmkuhl, 2004). However, this is only true when disturbances are strong enough to cause fatal damage to sensitive species (Rolstad et al., 2001). Some results show that low-levels of disturbance can even increment species richness in woodlands (Wohlgemuth et al., 2002). Most studies done have only considered the effect of high-intensity land-use and were performed in temperate and boreal climate areas (Coxson et al., 2003; Hedenas and Ericson, 2003). Therefore, the effects of low-intensity land-use are not well characterized. One important question left open is how low-intensity land-use affects the biodiversity, and especially what levels of disturbance are still favourable for species richness and when they become unfavourable.

The regional and local effects of three main global change drivers, chemical air pollution, climate change and eutrophication (Rockstrom et al., 2009b) remain unclear, including for Mediterranean-type ecosystems . All global climate forecasts predict some extent of climate changes, although predictions vary according to the region. For Mediterranean areas, an increase in drought conditions is expected, due to higher temperature and reduced precipitation (IPCC, 2007). As a consequence of this warming, an advance in many phenological events was already found for Mediterranean plant species, including leaf unfolding, flowering and fruiting and growing season (Gordo and Sanz, 2009). Climate change is also predicted to affect many ecosystem services, including water shortages, increased risk of forest fires, northward shifts in the distribution of typical tree species, and losses of agricultural potential, which in Europe will be most significant in Mediterranean areas (Schroter et al., 2005). Measuring the effects of climate change is normally done using long climate data-series (IPCC, 2007). A short-term way to find suitable ecological indicators of climate change, especially when looking at early signs of change, is to look for the effects in space under a climate gradient, because a spatial pattern may contain more information than a single point in a time-series (Dakos et al., 2010; Donangelo et al., 2010; Scheffer et al., 2009). Regarding air pollution, it is known that it has a strong effect in biodiversity and ecosystem functioning (Lovett et al., 2009; Paoletti et al., 2010). An additional concern is the direct link between atmospheric pollution and human health (MEA, 2005a). For example, particles under 25μm cause 800 000 premature deaths per year while exposure to occupational airborne particulates accounts for c. 300 000 deaths per year (Rockstrom et al., 2009a). In what concerns eutrophication based on atmospheric reactive N, it is known that, since the beginning of the 20th century, the industrial conversion of inert to reactive nitrogen allowed to make fertilizers and consequently a large human population growth (Erisman et al., 2008). Currently, most of the nitrogen entering the nitrogen cycle is of anthropogenic origin, from fertilizers, cattle and fuel burning (EPER, 2004; Galloway et al., 2003). Of this nitrogen used in human activities, about 75% is utilized in agriculture activities (Galloway et al., 2003), 60% of which is released to the environment, a large portion as atmospheric ammonia (NH_3) (Erisman et al., 2008). This nitrogen causes changes in terrestrial and aquatic ecosystems and on global geochemical cycles (Bobbink et al., 2010; Erisman et al., 2008; Gruber and Galloway, 2008). As a consequence it has caused species loss, replacing oligotrophic by nitrogen-tolerant ones (Bobbink et al., 1998; Lameire et al., 2000), which has been

probably occurring for decades (Dupre et al., 2010). An additional concern is that the interaction between two or more global change drivers may amplify the effects by synergies, or reinforcing feedbacks. From this interaction an uncontrolled cascade of effects may occur, such as the interaction of climate change and the historical drivers that cause species extinctions (Brook et al., 2008) or the interaction between pollutants and climate change that is a main concern in the Mediterranean area (Mentzakis and Delfino, 2010). These interactions may change the proposed thresholds for the planetary boundaries, because the combined effect of two global change drivers may be stronger than their sum (Rockstrom et al., 2009b).

Evaluating the effects of global change: the need for ecological indicators

Monitoring the global change drivers is naturally a major need. However, knowing its effects on ecosystems, including humans, is as important. For example, one may assess the increasing interference with the nitrogen cycle (Rockstrom et al., 2009b) calculating the nitrogen fixed every year globally by the industrial conversion of the atmospheric nitrogen by the Haber–Bosch process (Erisman et al., 2008). However, the effect of this nitrogen in ecosystems is far more than a simple increased concentration. On a practical perspective one is more interested in knowing if it has a meaningful effect on ecosystems diversity or functioning (Turnhout et al., 2007). In fact, a large fraction of the nitrogen is not used for food production, but leaks to the environment, causing major changes in terrestrial and aquatic ecosystems and on geochemical cycles (Bobbink et al., 2010; Erisman et al., 2008; Gruber and Galloway, 2008). Monitoring these changes in ecosystems is therefore crucial in order to know, mitigate or even anticipate their negative effects. However, ecosystem functioning is extremely complex and thus monitoring the effects of global change factors in ecosystems on an integrative perspective can be too time and resource consuming. As an alternative, scientists and environmental managers make use of ecological surrogates, named ecological indicators (Niemi and McDonald, 2004).

The European Environment Agency has a core set of environmental indicators that intends to comprise most environmental issues including indicators for air pollution, biodiversity, energy and transports, among others (EEA, 2005). A broad definition of environmental indicator is "a component or a measure of environmentally relevant phenomena used to depict or evaluate environmental conditions or changes or to set environmental goals" (Heink and Kowarik, 2010). Accordingly, ecological indicators are measurable characteristics of the structure, composition, or function of ecological systems (Niemi and McDonald, 2004), and are frequently used as a way to provide a link between decision makers and scientists. The ultimate goal of using ecological indicators is to be able to communicate complex issues in a relatively simple way (Niemeijer and de Groot, 2008). The information obtained by using ecological indicators can be used to predict future environmental changes and help identifying possible ameliorating actions, not only in the presence of anthropogenic disturbances but also in situations of natural alterations. For clarity within this thesis the term "ecological indicator" will refer to any measure made on the biological organism, the response variable, and it will be used in the sense of a state indicator within the DPSIR framework (Smeets and Weterings, 1999). The term "environmental factor" will be used referent to a stressor, a measure made on one or several environmental variables such as a pollutant concentration or climate, and will be used in the sense of a pressure indicator (Smeets and Weterings, 1999).

The use of ecological indicators to assess the state or current conditions of the ecosystem presents several advantages and limitations when compared to chemical analysis. Ecological indicators integrate the effect over time; that has advantages over the short time-limited chemical analysis; however the exact time of exposure that caused the effect is seldom known. Ecological indicators allow us to observe the effect of substances whose presence were not previously known and were therefore

never analyzed; but they rarely allow the identification of that substance. Moreover ecological indicators show us the effects of complex phenomena, whose individual characteristics would be very difficult to sample; one example is the effect of forest fragmentation that includes a complex array of changes associated to edge effects (Laurance, 2008). Ecological indicators are usually affected simultaneously by several environmental factors; this makes them comprehensive indicators of effects on the ecosystems, but complicates the separation of each factor. Finally, when using ecological indicators is not always obvious where to define a limit above which to consider a site to be polluted; thus legislation based on the values of ecological indicators is not frequent. Therefore, the use of ecological indicators cannot replace chemical analysis; but it can be used as a complementary approach that is fast and economic, and allows us to i) focus the chemical analysis on the critical areas; ii) be aware of even the smallest effect and thus use them as early-warning tools; iii) make sure that any change detected has a biological perspective, i.e. if the effect was biological meaningful (Pinho et al., 2004).

In general, ecological indicators should present a set of characteristics so that their application and interpretation is as straightforward as possible. These characteristics include not only responsiveness and measurability traits but also attributes related to usefulness (Dale and SC, 2001; Niemeijer and de Groot, 2008). Accordingly, ecological indicators should: i) be easily measured; ii) respond to stress, either of anthropogenic or natural origin, in a predictable way; iii) present a low-variability of response; iv) be robust, thus relatively insensitive to sources of interference; v) be comprehensible, easily understood by the target audience; vi) be compatible with indicators used in other regions; vii) be spatial and temporally bounded; viii) have thresholds that can be used to determine when management actions are necessary. Although a general framework for choosing the best indicator has been proposed by some authors (Howe et al., 2007; Niemeijer and de Groot, 2008), most authors agree that the choice of the indicators depends on the question under study (Dale and SC, 2001; MEA, 2005b; Niemi and McDonald, 2004). The difficulties on the use of ecological indicators derive not only on the complexity to fulfil these characteristics but are also enhanced as the complexity of the system being studied increases. Complexity in using ecological indicators may come not only from increasing the area of interest but also by increasing the level of biological organization to be monitored. This occurs when studying larger areas and when a higher number of indicators is being monitored, because the number of interactions between stressors and state indicators will also increase (Niemi and McDonald, 2004).

Among the large number of ecological indicators available, such as butterflies, carnivores, mussels, or plants (EEA, 2005) those that are sessile present the advantage of being easier to relate in a spatial explicit way to the underlying environmental factors. The effect of global change factors such as climate change and eutrophication in terrestrial ecosystems is directly mediated by changes in the atmosphere, including climate alterations and pollution concentrations in the air. Thus, it seems most appropriate to use ecological indicators that are as dependent on the atmospheric processes as possible. Most vascular plants only depend indirectly on the atmospheric conditions, because they use underground water and nutrient supply. Unlike those plants, bryophytes and lichens (but also many tropical epiphytes) rely entirely on the atmosphere for water and nutrient supply, thus being directly influenced by any change in the atmosphere. In Mediterranean-type ecosystems, lichens are ubiquitous and can be found all year-round on all habitats.

LICHEN AS ECOLOGICAL INDICATORS

Main characteristics of lichens symbiosis

Lichens are symbiotic organisms composed of one fungi partner and a photosynthetic partner, green algae, cyanobacteria or both (Honegger, 2009). The outcome of this symbiosis is a unique organism, which grows as a lichen thallus. From a nutritional

perspective lichens are known as "the symbiotic phenotype of nutritionally specialized fungi, ecologically obligate biotrophs which acquire fixed carbon from a population of minute photobiont cells" (Honegger, 1991). In these fascinating organisms, the fungal partner provides water, mineral nutrients and a protective structure and the photosynthetic partner provides photosynthates (Honegger, 1991). The idea that lichens are a symbiosis has been disputed and many see lichenization as a control parasitism (Ahmadjian, 1993) on which the fungus controls the algae population in order to maximize the fungi fit (Hyvarinen et al., 2002). However, the relation between the fungi and the photosynthetic partners is recognized to be rather dynamic (Richardson, 1999) and it is notorious that, when together, the partners may colonize areas that are out of the ecological range of either partners. In fact, lichens can colonize most substrata, including man-made structures. However, most lichens occur on plants (as epiphytes), on rocks or on soil. Lichens are the dominant life-form in 10% of the terrestrial ecosystems, those where vascular plants are at their physiological limits: high alpine, artic, antartic and dry ecosystems (Honegger, 2009).

Lichenization is a very successful strategy, with more than 20% of all known fungal species being lichenized (Honegger, 1991). Lichens name is given by the fungus, which represents in most species the largest proportion of lichen biomass. Lichenization occurs in many fungi taxonomical groups, although c. 99% of the 14000 lichens species known are ascomycetes (Honegger, 2009). The algae involved in the symbiosis are less diverse, mostly green-algae belonging to the genus *Trebouxia or Trentepohlia* (Fig 3 A,B,C) and the cyanobacterium to the genus *Nostoc* (Fig 3 D). In some species both green-algae and cyanobacteria coexist in the same thallus, being the green-algae the dominant partner and cyanobacteria restricted to special structures called cephalodia (Fig 3 A). One important characteristic of cyanobacteria is the capacity of nitrogen fixation increasing this way the nitrogen available for lichen growth (Honegger, 1991). A single lichen thallus may include and support a large number of other life-forms, including pro- and eukaryotic epi- and endobionts, which can be symptomless or parasitic, lichenivorous invertebrates, gall-producers and epibiotic bacterial films, among other for a revision see Honegger (2009). For this reason, lichens are considered consortia with an unknown number of participants (Honegger, 2009) or even mini-ecosystems (Purvis, 2000).

Lichen thalli develop in a variety of shapes, or growth-forms. These growth-forms include crustose (Fig 3 C), foliose (Fig 3 A,D) and fruticose (Fig 3 B), although some more specific designations exist, such as leprose, squamulose or umbilicate (Nimis and Martellos, 2008). Like other fungi lichens produce sexual fruiting bodies, mainly apothecia and perithecia, from which spores are dispersed. Once germinated these spores must combine with a compatible photosynthetic partner. Besides sexual reproduction lichens reproduce asexually by dispersing vegetative propagules that include both fungi and photobiont, most frequently as soredia and isidia.

Lichens can endure conditions that are lethal for most organisms. Examples such as the *Xanthoria parietina* that survived the preparation and observation under an electron microscope (Purvis, 2000) or the experience during which thalli of *Rhizocarpon geographicum* and *Xanthoria elegans* were exposed to space conditions including vacuum and solar radiation and once returned to Earth could regain normal metabolic activity (Sancho et al., 2007), highlight the extreme capacity of lichens species to withstand extreme environmental conditions when metabolic inactive. However, when lichens are metabolic active, they can be very sensitive to environmental changes. In this case a rank of species, from the most sensitive to the more tolerant, can be established. For these reasons lichens have been successfully used as ecological indicators.

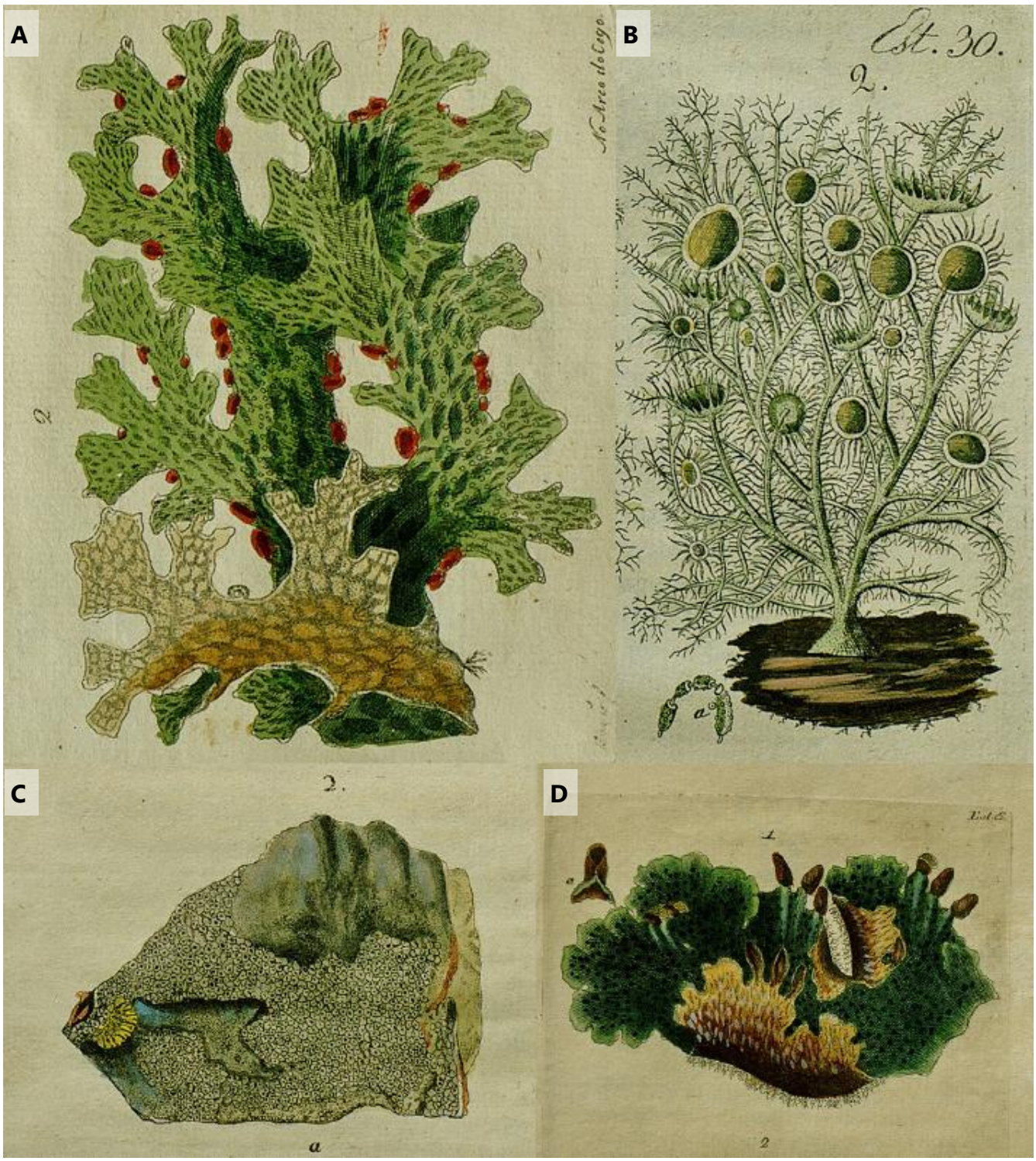

Fig. 3: Lichens ilustrations from an historical publication on lichens by G. Hoffman in 1800. **A)** "*Pulmonaria reticulata* Hoffm. (1789)" *Lobaria pulmonaria* (L.) Hoffm. 1796, **B)** *Usnea florida* (L.) Weber ex F.H. Wigg. (1780), **C)** "*Patellaria flavovirescens* (Wulfen) Hoffm., (1790)" *Caloplaca flavovirescens* (Wulfen) Dalla Torre & Sarnth. 1902 and **D)** *Peltigera venosa* (L.) Hoffm. (1789). Names in quotes are the original names given by the author (Hoffmann, 1800) and current name is given without quotes.

Lichens present a number of physiological characteristics that makes them well suited as ecological indicators of particular atmospheric conditions (air pollution and climatic conditions). Because lichens are poikilohydric their water content equilibrate constantly with the surrounding atmospheric humidity conditions and they are capable of using different sources of water, including precipitation, dew and fog (Hartard et al., 2008). Lichens do not have roots or a protective

layer isolating them from the surrounding environment, such as the cuticle of vascular plants see Honegger (2009). For this reason, lichen can absorb both nutrients and pollutants directly from the atmosphere. Because they are a symbiotic organism, damage to any of the partners results in losses to the entire individual. Thus, any disturbance lethal to one of the partners will cause the loss of the entire individual. Additionally, lichens are ubiquitous on land ecosystems and can be collected and identified throughout the year, which makes them a reliable indicator.**The history of lichens as ecological indicators**

The responsiveness of lichens to changes in the environment can be monitored by measuring i) variations in lichen diversity and/or abundance; ii) concentrations of accumulated pollutants or other substances; and iii) changes of physiological parameters (Branquinho, 1991). This thesis will be focused on the first two strategies.

Lichens are very frequently used to study the spatial and temporal trends of atmospheric pollutants or other substances. For this purpose, either lichens growing *in situ* or transplants of lichens from a control area can be used (Conti and Cecchetti, 2001). Lichens have been used as accumulators of heavy metals, essential nutrients such as sulphur, phosphorus and nitrogen, complex organic compounds and radionuclides (Augusto et al., 2007; Augusto et al., 2009b; Branquinho et al., 1999; Branquinho et al., 2008; Garty et al., 1977; Garty et al., 2009). This could be done because the concentration of these substances in lichens could be related to the concentration measured in the air, such as for copper (Branquinho et al., 1999) and NH_3 (Frati et al., 2007). Bryophytes, which although being completely separated taxonomically from lichens share with them many physiological characteristics, are also used frequently as accumulators of pollutants (Figueira et al., 2009). The use of lichens as accumulators can also be used simultaneously to the use of lichen diversity, in order to reinforce the indicator capacity to detect meaningful environmental changes.

The use of lichen diversity as an ecological indicator has a long history, since the work of Nylander in 1866 who proposed that the absence of lichens in the edge of the Jardins du Luxembourg was due to the incoming air pollution from the city (Gilbert, 1973) and suggested the usage of lichens as indicators for the "health of the air" (Hawksworth, 2002). This first approach to ecological indicators made use of the total lichens species richness: the higher the total species richness the better was the "health of the air", being the "lichen desert" the worse areas. Latter it became apparent that SO_2 was the key factor influencing lichen diversity. Accordingly, a semi-quantitative scale for estimating SO_2 concentration from epiphytic lichens diversity was developed (Hawksworth and Rose, 1970). This scale took into account that the different species present different sensitivities to SO_2 thus the more sensitive species disappeared at low concentrations and the more tolerant ones remained. For example, the presence of species such as *Teloschistes flavicans* (Sw.) Norman or *Lobaria scrobiculata* (Scop.) Nyl. was associated to "pure" air conditions, with SO_2 concentrations being below 30 μgm^{-3}. Later on, this approach gave rise to the IPA, Index of Atmospheric Purity (LeBlanc and De Sloover, 1970). To calculate this index each species was given a toxiphoby value (the degree of sensitivity to a given environmental stressor). The IPA value for each site was then calculated by considering each species abundance or frequency and its toxiphoby value (Kricke and Loppi, 2002). The more critical aspect of this approach was the calculation of the toxiphoby value. Although some attempts were made to standardize this calculation, with more than 20 formulas available, such as considering the more frequently accompanying species (Conti and Cecchetti, 2001), this method was never out of criticism. The main reason for this was the inclusion, in the same unique index, of species with contradictory responses to the stressor of interest (Kricke and Loppi, 2002). A striking example of this contradictory response was the species *Lecanora conizaeoides* Cromb. that has maximum abundance in intermediate polluted areas (Gilbert, 1973). A subsequent approach relied on the use of a reduced species pool, on which species with poor indicator

ability or with contradictory response to the stressor were removed. This index became known as the IPA_{18} because it made use of 18 species and was broadly applied on the country for which it was developed, Switzerland (Kricke and Loppi, 2002). This approach was naturally dependent on the species pool of the country on which it was built. The Swiss method was latter developed into a guideline for Germany that made use of standard sampling conditions and a sampling grid for estimating lichen frequency (Kricke and Loppi, 2002). A similar method was developed and applied in other countries, more extensively so in Italy where it become a national method for monitoring the effects of atmospheric pollution (Kricke and Loppi, 2002), after an important correlation was found between lung cancer mortality and lichen diversity (Cislaghi and Nimis, 1997).

Lichens as ecological indicators: from species to functional groups

When a single species is considered an ecological indicator, or "indicator-species", it is assumed that a single species represents the response of many more species with similar ecological requirements. A similar reasoning is the base of other approaches, such as using "umbrella-species" (a species whose conservation causes the conservation of many others) or "flagship-species" (a species with large public appeal) (Niemi and McDonald, 2004). Other approaches have been suggested, such as the construction of indexes that result from the sum of small selected subsets of the community. However, many authors point out critics to both species and indexes approaches, which included the large difficulty in linking the presence of abundance of species to key aspects of habitat quality and functioning and the perspective that indexes are an oversimplification and over generalizations of complex biological systems that do not take the processes into account (Niemi and McDonald, 2004; Suter, 1993).

The use of some form of indicator-species is not a robust indicator and thus does not facilitate one of the key aspects on the use of ecological indicators: to be able to separate the effect of the environmental change of interest from background variability. One of the main options available to do so is to aggregate data from lower taxonomic levels into groups (Niemi and McDonald, 2004). Most authors agree that aggregating species together results in more precise and reliable ecological indicators. This occurs due to the overlap of ecological amplitude of many species being smaller than the ecological amplitude of a single species (Diekmann, 2003).

The use of total species richness, the total number of species, has been frequently used as an ecological indicator in different taxonomic groups, although mostly to describe the conditions of the group itself (i.e. as an indicator of biodiversity), rather than to infer ecological effects (Carvalho et al., 2002; MEA, 2005a; Niemi and McDonald, 2004; Sim-Sim et al., 2000). Nevertheless, a number of critiques have been pointed to total species richness as ecological indicator, mostly related to the fact that all species have the same weight. This indicator does not differentiate among species regarding their sensitivity or resilience to environmental factors, nor takes into account key species for the ecosystem functioning or if species are native or not (MEA, 2005a). Moreover, different species may present opposing responses to the same environmental factor (MEA, 2005a).

Functional groups are groups of species with a similar response to an environmental factor (functional response groups) and groups of species with a common effect on ecosystems function (functional effect groups) (Lavorel and Garnier, 2002). They derived from the concept of guild, which is a group of species that uses the same type of environmental resource on a similar way (Root, 1967). It was observed that the number of functions represented in an ecosystem could be more important than species richness in determining the ecosystem processes (Tilman et al., 1997). Guilds have already been explored as ecological indicators to study the effects of forest management (Verner, 1984); based on the recognition that plant biological characteristics are related to the main environmental gradient present in the ecosystem

(Grime, 2001). Thus, functional groups could have a major advantage over species richness as ecological indicators, because they could be mechanistically linked to the environmental factor of interest and provide a detailed but still robust response as ecological indicators (Boutin and Jobin, 1998; McIntyre et al., 2003; van Diggelen et al., 2005). Although functional groups have been used as an ecological indicator, a considerable amount of work remains to be done. In fact, in order to use functional groups it is required to find the functional traits that respond to the stressor of interest (Cousins and Lindborg, 2004).

When compared to vascular plants, for which a large number of traits associated to important response and functioning is available (Diaz et al., 2004), far less is known regarding lichen traits related to responses to environmental factors (Stofer et al., 2006). The reason for this is that there is much less data on lichen than on vascular plants regarding the effect of environmental factors on key species characteristics such as competitive ability or growth rates (Stofer et al., 2006). Nevertheless, some lichen traits are available, more specifically morphological and reproductive ones, mostly from lichen floras. Additionally, expert knowledge classifications of lichens into response functional groups have been developed. These include the ones made for North America (McCune and Geiser, 2009), United Kingdom (Stofer et al., 2006), central Europe (van Herk, 1999) and Italy (Nimis and Martellos, 2008). This last classification, developed for Italy, includes lichens species with Mediterranean and more northern distribution, thus including most species present in other Mediterranean areas.

The Italian classification of lichens regarding their traits based on expert knowledge and habitat preferences (Nimis and Martellos, 2008) is available online (http://dbiodbs.univ.trieste.it) and includes for each species the information on morphology and reproduction traits: growth form (including 13 categories), photobiont (4 categories, green algae other than *Trentepohlia*, *Trentepohlia*, filamentous cyanobacteria, coccaceous cyanobacteria) and main reproduction strategy (4 categories, sexual, soredia, isidia, asexual). It also includes a number of ecological traits on which each species is classified from 1 to 5 regarding its maximum and minimum tolerance to the pH of the substratum (from very acid substrata to basic substrata), solar irradiation (from very shaded situations to very high direct solar irradiation), aridity (from hygrophytic to very xerophytic) and eutrophication (from no eutrophication to very high eutrophication).

Lichen traits associated to responses to environmental changes

Lichen photobiont type could be a trait strongly involved in species response to several environmental factors. Cyanolichens are known to differ in water requirements when compared to green-algae ones. Cyanolichens require liquid water to start photosynthesis (Green et al., 2002; Lange et al., 1986), and upon rehydration take longer to achieve maximum photosynthetic rate than green-algae ones (Palmqvist, 2000). For this reason, ecological performance of cyanolichens should be sustained by longer hydration time, which makes them usually associated to moister and shadier habitats (Maguas et al., 1997). Additionally within the green-algae group, we may also find a gradient of response to climate. In fact, the lichens with *Trentepohlia* as photobiont seems to be favoured in relation to other green-algae lichens regarding the possibility of reinvasion of areas previously polluted in Europe (Aptroot and van Herk, 2007). This was suggested to be due to the better adaptation of *Trentepohlia* lichens to the ongoing climate change (Aptroot and van Herk, 2007), once these lichens dominate in tropical ecosystems. Besides determining the response of lichens to climate factors, the photobiont can also influence the response to human related disturbances, such as pollution. In fact cyanolichens were shown to have disappeared from polluted areas throughout Europe, in particular those subjected to acid rain (Richardson and Cameron, 2004). In California, cyanolichens were nearly absent from areas with more agriculture land-use (Jovan and McCune, 2004). However, there is a surprisingly scarce

number of publications linking the trait to the community distribution in the field (Jovan and McCune, 2004) and specially to the quantification and modelling of that relations.

Growth form is a trait that influences the capability of lichens to intercept and thus accumulate pollutants such as metals and organic compounds (Augusto et al., 2009a; Bajpai et al., 2010) and its is accepted that the amount of a pollutant found in lichens is species-dependent (Wolterbeek et al., 2003). Despite different growth forms accumulate different amounts of pollutants (Bergamaschi et al., 2007) and different growth forms show different intensity of effect regarding biodiversity (Perlmutter, 2010), all lichen species will show an measurable effect. This was shown for example on the use of lichen diversity as ecological indicators of the effect of land use intensity (Bergamini et al., 2005), with both crustose and macrolichens showing good correlations with land-use intensity.

The reproduction strategy was also shown to influence lichens species response to environmental factors. Species mainly reproducing sexually have higher relative abundance in areas with more intense human occupation that the ones reproducing mainly asexually (Stofer et al., 2006) and sexually dispersed species arrive first to disturbed forests than asexually dispersed ones (Hedenas and Ericson, 2000). Additionally, sexually reproducing species prevailed over asexually reproduction on the proximity of a copper smelt (Mikhailova, 2007). Thus sexually reproducing species seem to be more tolerant to environmental changes than asexually reproducing ones.

Lichens can also be classified accordingly to their preferences or tolerance to eutrophication (Nimis and Martellos, 2008). Several authors have pointed out that nitrogen from agriculture origin is playing an increasing role in changing lichen communities (Hultengren et al., 2004; Purvis et al., 2003). Distance to farms (van Herk, 1999) and presence of cattle grazing areas (Ruoss, 1999; van Herk, 2001; Wolseley et al., 2006) has been tested and confirmed as a significant cause of change in lichen communities. Moreover lichens have been used to monitor the effect of NH_3 at both large (Sparrius, 2007; Sutton et al., 2009; van Herk et al., 2003) and small spatial scales (Frati et al., 2007; Wolseley et al., 2006). However, not only nearly all these works were done for temperate areas but also large problems remain due to the presence of multiple environmental factors (Giordani, 2007; Jovan and McCune, 2006). These problems prevent the use of lichens as ecological indicators of the effects of eutrophication at large spatial scales on a consistent and reliable way. The traits associated to this tolerance remain under study. When comparing the cation exchange capacity between a nitrophytic (*Xanthoria parietina*) and a non-nitrophytic species (*Flavoparmelia caperata*) other authors found that *X. parietina* has a much lower cation exchange capacity compared to the non-nitrophytic species *F. caperata* (Gaio-Oliveira et al., 2001). Moreover *X. parietina* presented always higher nitrogen concentration than *F. caperata*. The authors suggested that the lower cation exchange capacity of the nitrophytic species could protect it from higher nitrogen concentration in the environment (Gaio-Oliveira et al., 2001). In fact it was found that only under very high ammonium availability the nitrophytic lichens species *X. parietina* showed a negative response (Gaio-Oliveira et al., 2004; Gaio-Oliveira et al., 2005). The change in the substrata pH was another mechanism that has been suggested to be involved in eutrophication tolerance, or more specifically to NH_3 tolerance (van Herk, 2001). Although of great interest, the use of these ecological traits (e.g. cation exchange capacity) is not feasible because it would imply to test all species in all environmental conditions found. Therefore, a more practical approach is to use an expert knowledge classification of lichens into species based on its habitats preferences and to test this classification whenever possible.

Standardization of sampling methods: a step towards universal indicators

In order to make lichens accepted as ecological indicators one important step is to standardize methodologies. The most recent achievements on

standardizations have been done on the sampling design phase (Cristofolini et al., 2008). One of those achievements was to recognize that is was desirable to homogenise the effects of unwanted environmental factors so that the alterations of the lichens data could be attributed to the environmental factors of interest to the study (Pinho et al., 2004). One important example is the preferential use as ecological indicator of epiphytic lichens growing on the main tree trunk. In fact lichens growing on rock or soil are strongly influenced by site land-use and those growing on twigs are strongly influenced by tree crown architecture. The main tree trunk is more likely to be similar between sampling sites and thus more easily comparable.

Another important achievement of the most recent work in the use of lichens as ecological indicators has been the establishment of a standardized methodology for collecting lichen diversity and abundance data in the field, called the "European Method" (Asta et al., 2002). This methodology is based on a sampling grid, with 50x10cm, divided into 5 squares, that is placed on the four main aspects of tree trunk (Fig. 4). All lichens species occurring within the grid are identified and the number of squares each species occurs in each tree is recorded as their frequency. From this data one can calculate two main variables: total species richness and total LDV, Lichen Diversity Value (Fig. 4). The Lichen Diversity Value is a measure proposed in the protocol, and is the sum of the frequency of all species in each tree, divided by the number of trees sampled. This protocol is starting to be more frequently applied in Europe and United States (Giordani, 2007; Perlmutter, 2010; Svoboda et al., 2010). Nevertheless, although standardization of the sampling methods may allow a more easy comparison of the results between different regions, there is still a large quantity of work to be done regarding the interpretation of data, especially on the interpretation of the effects of multiple environmental factors on lichen variables.

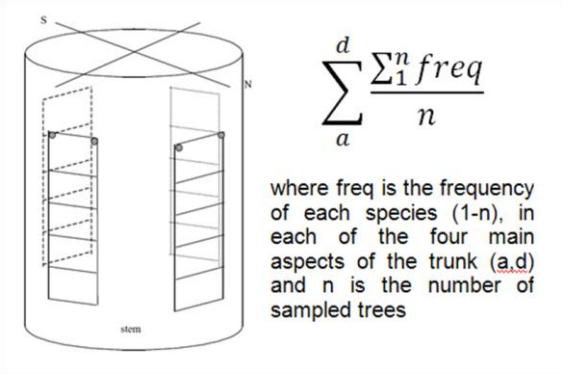

Figure 4: Distribution of the sampling grid in a tree trunk, original picture (Asta et al., 2002).

Lichens as ecological indicators in a spatial and multiple factors context

One important step of using ecological indicators is the possibility to relate them to the underlying environmental factors of interest. However, ecological data is often the result of factors working at different spatial scales (Maurer, 2002). As a result, the environmental factors are more or less superimposed in the territory, and not always influencing the entire study area (Fortin and Dale, 2005). This greatly complicates the interpretation of the ecological indicators data. Therefore, in order to account for this problem, the data interpretation should take into consideration the different possible scales of analysis.

Since the late XIX century SO_2 was the prevailing pollutant on Europe and thus lichen diversity could be used as ecological indicator of the effect of SO_2 in a straightforward way (Hawksworth and Rose, 1970). However, since the 1980 the world emissions of SO_2 have been declining (Stern, 2006) a decline that has been very large in North America and Europe (Smith et al., 2001). As a consequence, it has become apparent that factors other than SO_2 can also negatively influence the ecosystems. The rise in importance of environmental factors other than SO_2 has also been detected in lichen communities (Purvis et al., 2003) and the use of lichens as ecological indicators has shifted from monitoring the effect of SO_2 and associated pollutants to a more general framework that includes not only

monitoring the effect of air pollution (Geiser and Neitlich, 2007; Giordani et al., 2002), but also the effect of more complex and subtle environmental changes such as habitat fragmentation, habitat stability and influence of forest management (Coxson and Stevenson, 2007; Edman et al., 2008; Nascimbene et al., 2007; Ranius et al., 2008). Although lichens have been successfully used as ecological indicators of a large number of environmental changes, one common problem was raised in most studies: the influence of multiple environmental factors that work in the same territory, making the results more difficult to interpret. Most authors report that along with the influence of pollutants other factors such as bark texture and moisture (Perlmutter, 2010), forest age (Svoboda et al., 2010) and also climate (Garcia et al., 2005; Giordani, 2007) influence lichen diversity and thus limit its use as ecological indicator. How to separate the effects of these multiple environmental factors, remains one of the major challenges of using lichens as ecological indicators (Dale and SC, 2001; Niemi and McDonald, 2004).

Land-cover types could be used as a surrogate for multiple environmental factors. Although each land-cover type may emit different amount and types of pollutants, only few authors have tried to establish a linkage between land-cover types, the associated emission of pollutants and their effects on ecosystems (Frati et al., 2006; Gombert et al., 2004; Jovan and McCune, 2005; Purvis et al., 2003). Some authors have used land-cover as a surrogate of pollutants emission, and relate neighbourhood land-cover to ecological-indicators data (Aarrestad and Aamlid, 1999). Others authors (Tommervik et al., 1998) have explicitly considered land-cover as a potential source of pollutants, taking into account the distance from these to ecological-indicators. However, most works considered only urban and/or industrial land-cover types and usually only one fixed distance to calculate the neighbourhood (Augusto et al., 2004; Frati et al., 2006; Gombert et al., 2003). In Mediterranean-type ecosystems, the influence of nearby agriculture areas, even of low intensity must also be considered (Branquinho et al., 2008; Loppi and Pirintsos, 2000).

One powerful family of methods to analyze and interpolate spatial structured data is geostatistical analysis. The advantage of geostatistics methods results from the fact that they provide the development of the spatial model of the variable which is used in interpolation with known uncertainty (Soares, 2000; Store and Jokimaki, 2003). Furthermore, it may provide generalization and up scaling methods (Burrough, 2001). Geostatistical techniques are frequently used in the analysis and interpolation of biomonitoring data (Figueira et al., 2009; Gay and Korre, 2006; Pesch et al., 2008).

Geostatistical methods use the spatial variance of the sampled data to model the spatial structure of the data and ultimately interpolating the variable values in unsampled locations using kriging (Fortin and Dale, 2005). This process is based on variography analysis (Mitchell et al., 2000). An empirical variogram (Fig. 5) is calculated by plotting the semi-variance values (calculated between pairs of sampled points) against the spatial lag (Fortin and Dale, 2005). In a variogram of a variable with some spatial structure, for shorter distances the values of the semi-variance tend to be small (that is, points located nearby are more likely to have similar values). The distance between the interception at the origin and zero is the nugget effect, values closer to zero indicating a stronger spatial structure. The nugget effect is thus a measure of the effect of factors working at a scale smaller than the sampling grid and of the effect of non-systematic sampling errors (Soares, 2000), that is, variance without spatial structure. At higher distance the semi-variance values tend to increase reaching a plateau, the sill (Fig. 5). The distance at which the semi-variance reaches a sill is the range, which indicates the distance up to which the variable presents some a spatial structure. Many patterns of environmental variables present some degree of anisotropy, that is, two sites are likely to be more similar when located in a given direction. To incorporate this main-direction feature, a variogram is calculated in the direction of maximum spatial continuity and another to its perpendicular direction. A model describing the main spatial characteristics of the studied variable can then be fitted to the empirical variograms. This model can be used not only to

interpolate the variable to unsampled locations, but also to analyze and compare the spatial characteristics of the variables under study.

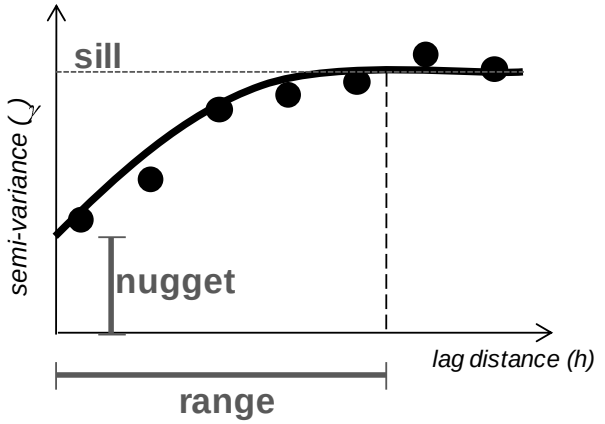

Figure 5: Theoretical example of a variogram analysis for a spatially structured variable. The nugget effect is a measure of the importance of factors without a spatial structure and the range the distance up to which the variable presents a spatial structure. The dark line is the model fitted to the empirical data describing the variable spatial structure.

AIMS & OUTLINE

The general aim of this thesis is to provide a framework for the use of lichen functional-diversity as an integrating ecological-indicator of environmental changes in Mediterranean type ecosystems. This will be supported by the analyses of the lichen community responses to environmental factors allowing the selection of lichen-variables as potential ecological indicators. The selected indicators can then be modelled against the key environmental factors, such as the ones associated to drivers of global change (eutrophication, climate and, air pollution). For that, a spatial explicit analysis will be used in order to disentangle the response of lichen communities to multiple environmental factors and ultimately to be able to apply the selected ecological indicators to monitor the complex Mediterranean-type ecosystems in a changing environment.

After a general introduction in chapter 1, the second chapter (divided into two sub-chapters: ch02.1 and ch02.2) aims to the improvement of the tools needed for the development of the proposed work, and was based on the fact that the selection of ecological indicators of environmental change can be done using spatial gradients instead of long-time series, since it allows the study of a full range of responses to the environment factors in a short period. Moreover, environmental factors work at different spatial scales, many superimposed in some areas, and the available studies dealing with the local effects of global change drivers lack spatial resolution and ground truthing. In this context, in **sub-chapter 2.1** we hypothesized that lichen diversity is affected by different environmental factors working in the same area and that we could be able to disentangle their effects by using spatial analysis. We considered epiphytic lichens total species richness and abundance, as well as neighbourhood land-cover. We used geostatistical tools to analyse the variables in space having into account: i) the area occupied by each neighbourhood land-cover type; ii) the distance of each land-cover type to lichens sampling sites and; iii) the spatial location of sampling sites in the study region.

The response of functional groups, assemblages of species with a similar response to an environmental factor, can be mechanistically linked to an environmental factor of interests. In **sub-chapter 2.2** we hypothesised that different functional groups might present different responses to the underlying environmental factors and could therefore be used to disentangle the effects of multiple environmental factors, in particular of some drivers of global change at local scale. We considered as environmental factors short and long-term indicators of microclimate, forest structure and density, and the area occupied by artificial areas in the neighbourhood of the sampling sites, as sources of disturbance. The ecology of lichens communities within a natural forest was then modelled in response to key environmental factors, using functional groups based on the main photobiont type.

Once lichen functional diversity has been related to the underlying environmental factors in a spatial explicit way in the previous chapters, it becomes necessary to characterize the spatial and temporal

characteristics of lichen-variables as ecological indicators. In **chapter 3**, we hypothesise that lichen functional groups based on the main photobiont type presented different spatial and temporal response patterns to a major environmental factor associated to the drivers of global change, climate alterations. To do so, we considered a climate gradient driven by orography and measured by potential solar radiation, and tested its effects on epiphytic lichens functional groups within a Mediterranean forest.

Most Mediterranean ecosystems are semi-natural areas with low intensity agriculture land-use, but the effect of this environmental factor on biodiversity is rarely studied. Moreover, the use of lichen variables based on functional diversity needs to be compared with other lichen variables based on total diversity. The **chapter 4** studies the influence of traditional low-intensity land-use in epiphytic lichens species and functional groups. We tested the hypothesis that ecological indicators based on eutrophication tolerance responded to low-intensity land-use. For that, lichens were sampled in a gradient of low-intensity land-use, which included forestry and pasture activities. A number of lichen variables were tested: total lichen species richness, total species abundance (LDV) and richness and abundance of functional groups, divided according to eutrophication-tolerance.

The effect of agriculture activities emerged from the previous chapters as a widespread environmental factor influencing all previously studied ecosystems. Agriculture activities are known to emit NH_3 whose effect on lichens species and functional groups is not well understood in Mediterranean-type ecosystems. Additionally, in these areas lichens could be most useful as ecological indicators of the effect of NH_3 due to the lack of national monitoring networks for this chemical and its effect in ecosystems. On the other hand, functional groups related to eutrophication tolerance are based on expert knowledge (Nimis and Martellos, 2008), which has never been explicitly tested under a NH_3 measured gradient for Mediterranean-type ecosystems. In **chapter 5** we aimed at understanding the isolated effect of atmospheric NH_3 in lichens species and functional groups and the implications of their use as ecological indicators of eutrophication and of land-use intensity. We also aim at testing the expert-knowledge classification of lichens into functional groups. For that we characterized lichen functional groups in a full-range gradient of NH_3 concentrations, measured in Mediterranean woodland for one-year period.

Taking into consideration the results from the previous chapter, it becomes important to determine the critical levels of NH_3 for Mediterranean woodlands, which was not known previously. The critical level is the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge (Cape et al., 2009). Calculation of NH_3 critical levels was done in **chapter 6** by determining the concentration of NH_3 above which lichen functional-diversity differed from background levels.

To fulfil the limitation of using lichen functional diversity to estimate the effects of atmospheric N pollution in a multiple factor context, where potential confounding factors might cause the same response in lichen community, an additional strategy was needed, namely the use of lichens as bio-accumulators of N. In **chapter 7** we hypothesise that total nitrogen in lichens could be related to the estimated NH_3 atmospheric emissions at regional level. This results could provide a link between the predictions made for nitrogen deposition, which are based in models, and the actual nitrogen deposition in ecosystems. To do so we related nitrogen concentrations measured in lichen thalli to N emissions estimated in two different ways. Additionally we used the spatial analysis tools developed on the previous chapters to map the areas at greatest risk, especially within Natura 2000 network.

In large regions, with patchy Mediterranean landscape, it is important to known the areas more affected by human disturbances, so that further environmental management could be focused on those areas. This challenging situation for the use of lichens as ecological indicators was addressed in

chapter 8. In this chapter the aim was to apply the knowledge obtained in the previous chapters in a complex Mediterranean region, with a large area (> 1500km^2) and a patchy landscape. This region presents multiple environmental factors that comprise both natural and anthropogenic disturbances, including scattered urban areas mixed with an agriculture land-use and also an important industrial area. In this chapter we made use of lichen functional groups, pollutants concentration measured in lichens and a spatial explicit analysis considering several spatial scales.

Finally, in **chapter 9** a general discussion is presented, integrating the key results from the previous chapters. The objective is to present and discuss a general framework for the use of lichen functional-diversity as an integrating ecological-indicator of the effect of global change drivers in Mediterranean-type ecosystems. We will discuss the selection of the lichen-variables that are more suitable as ecological indicators, including the methods available for disentangling the effects of multiple environmental factors. The potential use of lichens as early-warning ecological indicators and their use as universal ecological indicators will be further addressed.

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Chapter 2

02 | Tools for the development and analysis of ecological indicators

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2.1 | Impact of neighbourhood land-cover in epiphytic lichen diversity: analysis of multiple factors working at different spatial scales

ABSTRACT

The objective of this work was to determine the impact of neighbourhood land-cover in epiphytic lichen diversity. We used geostatistics to analyse the spatial structure of lichen-indicators (number of lichen species and Lichen Diversity Value) and correlate them to land-cover considering different distances from the observed data. The results showed that lichen diversity was influenced by different environmental factors that act in the same territory but impact lichens at different distances from the source. The differences in the distance of influence of the several land-cover types seem to be related to the size of pollutants/particles that predominantly are dispersed by each land-cover type. We also showed that a local scale of analysis gives a deeper insight into the understanding of lichen richness and abundance in the region. This work highlighted the importance of a multiple spatial scale of analysis to deeply interpret the relation between lichen diversity and the underling environmental factors.

INTRODUCTION

Among the potential biodiversity tools, lichen communities are one of the most commonly used in biomonitoring programmes. These biomonitoring studies have been done worldwide confirming that sensitive species decline in polluted areas, and tolerant species remain (Geebelen and Hoffmann, 2001; Giordani et al., 2002; Nimis et al., 1991; Pirintsos and Loppi, 2003; Vokou et al., 1999). Lichens are a symbiosis between a fungus and an algal partner (green algae or/and cyanobacteria (Honegger, 1991), with characteristics that makes them an appropriate model organisms to understand the relations between biodiversity and multiple environmental factors. The responsiveness of lichens to changes in the environment can be monitored by using variations in lichens diversity and/or abundance, using physiological parameters, or lichens as accumulators of pollutants (Branquinho, 1991).

Regional surveys are often performed in lichen diversity assessments and in biomonitoring studies. Most authors perform a global analysis of all sampling sites, expecting to extract one or more relations between lichen variables and environmental factors for the entire territory. However, in large regions multiple environmental factors are expected, some of them may be superimposed in the territory, which greatly complicates data interpretation. On the other hand these environmental factors affect living organisms at different levels or scales (Maurer, 2002) and consequently, scales influence our perception about any phenomena under study (Dungan et al., 2002; Heglund, 2002). In order to account for this we performed an analysis at two different spatial scales, resulting in a regional and local level of analysis.

The relations between biodiversity and environmental factors can yield much richer results if the importance of the spatial dependence of biotic responses is interpreted. As a result, several authors have recently become interested in examining spatial relations between lichen variables and environmental factors (Liebhold and Gurevitch, 2002). The relation of land-cover in the neighbourhood of sampling sites with pollutants concentration and lichen cover was assessed other authors (Tommervik et al., 1998), as well as the use of the distance to landscape elements as explanatory variables, such as distance to roads and farms (van Herk, 1999), traffic intensity and roads (Gombert et al., 2003).

It appears to be essential that approaches that take into consideration the importance of the disturbance sources, such as: distance, covered area, amount of pollution emissions, or other, are an added value for interpreting lichen data. However, there are no defined rational to perform this analysis. Several authors relate lichen variables with environmental variables that were only measured in the sampling site location (Aarrestad and Aamlid, 1999). Others authors used small distances from the sampling sites such as 200 m (Tommervik et al., 1998), to relate lichen cover with pollutants. Other authors have used larger distances such as 2 km for relating the land-cover with dioxins and furans measured in lichens (Augusto et al., 2004).

Spatial analysis of biodiversity data requires the use of geostatistical tools that have been showing to be important for vegetation ecology and biomonitoring studies. A geostatistical study of the spatial characteristics of variables alone can provide insight on biomonitoring studies through the interpretation of the variograms (Mitchell et al., 2000). The importance of geostatistics also results from the fact that it provides the possibility to develop a spatial model of the variable which is used in interpolation with known uncertainty (Soares, 2000; Store and Jokimaki, 2003). Furthermore, it may provide generalization and up scaling methods for other areas (Burrough, 2001).

The objective of this work was to determine the impact of multiple environmental factors in lichen diversity taking into account: i) the area occupied by neighbourhood land-cover class; ii) the distance of each land-cover class to lichens sampling sites; and iii) the spatial location of sampling sites in the study region. For that epiphytic lichen species richness and abundance as well as and neighbourhood land-cover classes considering many distance from sampling sites were measured and analysed at a regional and local level of analysis. We hypothesize that lichen diversity is affected by different environmental factors acting in the same territory and that those environmental factors influence lichens at different distances. We also hypothesized that most studied environmental factors act at the local spatial scale, having in consideration the nature of the major land-cover types with detrimental effect on lichens and the nature of particle/pollutants dispersed by those areas.

METHODS

Study region and sampling sites

The study region (c. 1500 km^2, 50x30 km) is located on the SW coast of Portugal (Fig. 1). It includes two small mountains (max. 383 m) parallel to the coast. Annual average temperature is between 16-17.5ºC, average annual precipitation 600- 1000 mm and annual insolation 3000 h, averages from 1931 to 1960, (IA, 2006). The prevailing winds are from the N-NW. The study region includes 4 municipalities: Grândola (population 14901), Santiago do Cacém (population 31105), Odemira (26106) and Sines (population 13577) (INE, 2001).

Most of the landscape is dominated by agro-forestry, mainly cork-oak woodlands (*Quercus suber* L.) and annual agricultural activities (mainly for grain production). Coastal areas present a more intense human occupation that includes cork-oak woodlands but also maritime pine (*Piunus pinaster* Aiton) and eucalyptus (*Eucalyptus* spp) plantations. This is an important industrial area established since the late 1970's with a coal power plant, an oil refinery, a chemical plant and, an industrial landfill, as well as, many other smaller industries. The sea harbour allows the arrival of raw materials such as coal, oil, gas and other chemicals. There are two main motorways and one railway (Fig. 1). Recently, there has been an increase of urban construction in the coastal areas.

Within the study region 71 sampling sites were randomly selected for assessing lichen-indicators, ensuring at the same time a higher density of sites near the industrial area, where most changes were expected. Sampling was done exclusively in un-harvested cork-oak trees within cork-oak woodlands, under constant low-intensity land-use. This very strict sampling criterion intended to minimize sources of intra-site variations, such as tree species or land-use intensity.

Data collection and calculation of lichen indicators

Lichen data were collected in early works (Pinho et al., 2004). Epiphytic lichens were assessed in trunks of *Quercus suber* L. trees using a standard methodology (Asta et al., 2002), only for foliose and fruticose species, using a grid with five 10x10 cm squares, placed according to the four main orientations of the trunk. All foliose and fruticose species found inside the squares were sampled, and the number of squares on which they occurred was recorded as their frequency. From this data total LDV (Lichen Diversity Value) and Total Number of Lichen Species (NrSp) were calculated. LDV accounts both for the number of species and their frequency (Asta et al., 2002).

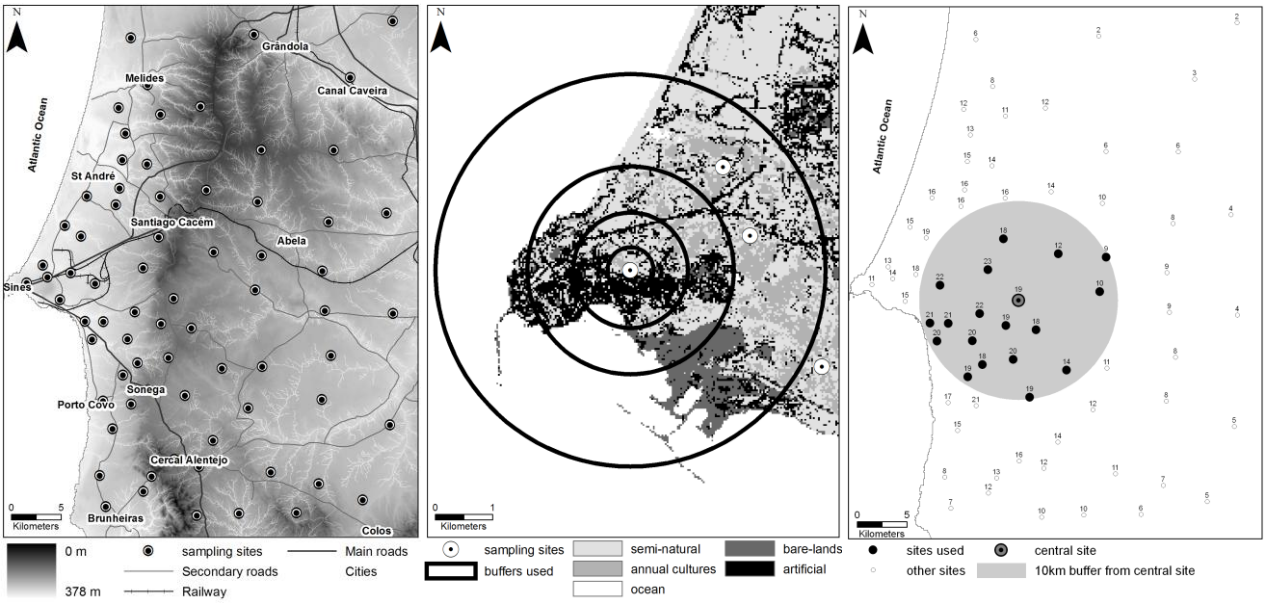

Figure 1: Left: Study region, main cities, roads and altitude; lichen-diversity sampling sites are also represented. Centre: Method for determining the area occupied by each land-cover class around each sampling site: the area was determined for several distances and used for regional correlation analysis (using all sampling sites, see Fig 1) and local correlations analysis (using groups of sampling sites). Right: Methods for local correlation analysis; it is shown an example for a site (named "central site"): a 10km radius is considered around each site, all sites within that radius are chosen to calculate the local correlation between LDV and land-cover; the number of sites (samples) used in each site are indicated by the number (ex. 19 samples in "central site").

Land-cover map analysis

The area consists mainly on cork-oak woodlands surrounded by many human-made land-cover types, and by ocean, resulting on a heterogeneous landscape. As a part of the Sinesbioar project (LIFE00 ENV/P/000830, project site at www.ccdr-a.gov.pt/sinesbioar), a land-cover classification with 9 classes was generated by assisted classification of Landsat images (30m resolution, multispectral) using a maximum likelihood algorithm (data not shown). The classification was validated using ground truth data. With this classification, it was possible to characterize the land-cover in the neighbourhood of each sampling site. After preliminary analysis of the land-cover classes regarding their influence on LDV and NrSp, 4 land-cover classes that showed a negative impact were selected: 1)"ocean" (all oceanic areas); 2)"bare-lands" (quarries and soil with little or without vegetation due to soil degradation); 3)"annual cultures" (annual cultures mainly for grain); and 3)"artificial areas" (urban, industrial and roads). Land cover-variables were calculated as the area (m^2) occupied by each land-cover class in the neighbourhood of sampling sites. Area values were log prior to the analysis.

Data analysis: distance of influence

In order to evaluate the influence of the neighbour land-cover classes in lichen-indicators, LDV and NrSp, we calculated the Pearson correlation coefficient (R) between the lichen-indicators and the area occupied by each land-cover class in the neighbouring of each sampling sites. We have used a circular neighbourhood centred in each sampling site (circular buffer around each sampling site) using a radius between 100 m and 6600 m (Fig. 1).

The 6600 m limit was shown to be sufficient to characterize the influence of most of the studied land-cover types (Fig. 3). Then, a correlation coefficient between each lichen-indicator and each land-cover class was calculated for each buffer size. The distance of influence for each land-cover class was considered the buffer size for which the maximum correlation coefficient was obtained. This assumption was based in the idea that, if the correlation coefficient increases as we enlarge the circle radius, then the new areas added are still affecting lichens. When the correlation coefficient becomes lower or similar we considered that those new added areas were no longer influencing biodiversity.

Local and regional correlations

The correlation between lichen-indicators and land-cover classes was performed at a regional level, using all sampling sites (Fig. 1). This was reported as regional correlations. The previous procedure was then repeated, but instead of considering all sampling sites, the correlation calculations were confined to "local areas", thus obtaining what we considered local correlations. In practical terms, correlation coefficients were calculated inside moving windows that visited all sampling locations (Fig. 1), obtaining a correlation coefficient of a lichen-indicator versus a land-cover class (ocean, bare-lands, annual cultures or artificial areas) for each sampling location, based on all samples located inside the "local area" centred in that specific location.

A routine for calculating local correlations was implemented in Fortran 90 using circular moving windows with variable sizes (CERENA, 2000). Several window sizes were tested and it was concluded that; i) windows with very large sizes (>10 km) tended to display results similar to the regional ones, since they included most of the study region; ii) windows with very small sizes (<10 km) displayed non-significant correlations due to the low number of samples inside the window; iii) a circular window with 10 km radius around each sampling site was considered the optimal window size from all tested, optimizing the statistical consistency of calculated R.

The number of samples used to calculate local correlations is therefore different for each sampling site (Fig. 1), ranging from 3 to 22. Significant correlations for $p<0.05$ were considered and mapped in the studied region using discrete symbols. Additionally, for each land-cover class the distance for which the maximum correlation coefficient was obtained (see MM, the distance of influence) was also mapped. This was done by interpolating those distances using the Kriging estimator available in ArcMap.

Other data analysis and software used

The spatial continuity of lichen variables was evaluated by variography analysis. For each variable we calculated experimental variograms and manually fitted a theoretical model, a linear combination of spherical models (Goovaerts, 1997) using GeoMS (CERENA, 2000). Regional correlations were performed with Statistica 7 (StatSoft, 2004) and all mapping operations and output figures were prepared with ArcMap 9 (ESRI, 2001). Data management was made using Exel 2003 and Access 2003 (Microsoft, 2003).

RESULTS

Spatial structure of data

The results of variography analysis of lichen-indicators (Fig. 2, Table 1) showed the absence of

nugget effect (C_0), which confirmed the insignificant influence of site variables as already observed in early works (Pinho et al., 2004). Although a null nugget effect is unlikely in biological systems, due to intraspecific variability, any value that had been chosen in this model (within the observed semi-variance for shorter distances) would not affect our results. Experimental variograms of LDV were fitted by a sum of two spherical structures (C_1,C_2,A_1,A_2) suggesting the existence of two groups of variables influencing LDV. The relative importance of each structure was given by the variance accounted for C_1 and C_2 and by each structure range A_1 and A_2 (Table 1). The variogram of NrSp indicated the existence of a single structure (C_1,A_1). The spherical models showed to be the best fitting to the experimental variograms. We observed high spatial continuity in the main direction: zonal anisotropy of LDV has been modelled by a large range of the second structure, (81 Km) exceeding the maximum length of the study region. We could also observe a strong anisotropy for LDV (Table 1), for which the main orientation was north, indicating that sites located along a North-South direction were more correlated than those located along a East-West direction. The variable NrSp showed a variogram range of 9000 m and was found to be isotropic (Table 1). The first structure, C_1, accounted for half of LDV variance and accounted for all the NrSp variance, reflecting the influence of small-range environmental variables. Such range was 9000 m for NrSp and 12500 m for LDV. The second structure of LDV, C2, represents the influence of the long-range environmental variables with more than 81000 m (Table 1).

Table 1: Spatial characteristics of the fitted function for each lichen-diversity variable. C_0: nugget effect; $C_{1,2}$: partial sill; $A_{1,2}$: range; anisotropy$_{1,2}$: main direction/minor direction; total C: total variance. All models were spherical.

lichen-diversity variable	C_0	C_1	A_1	anisotropy$_1$	C_2	A_2	anisotropy$_2$	total C	main direction
Total Number of lichen Species	0	14.00	9000	1.000	-	-	-	14.00	10º (±N)
Lichen Diversity Value	0	75.563	12500	1.136	39.291	81000	6.146	114.864	0º (N)

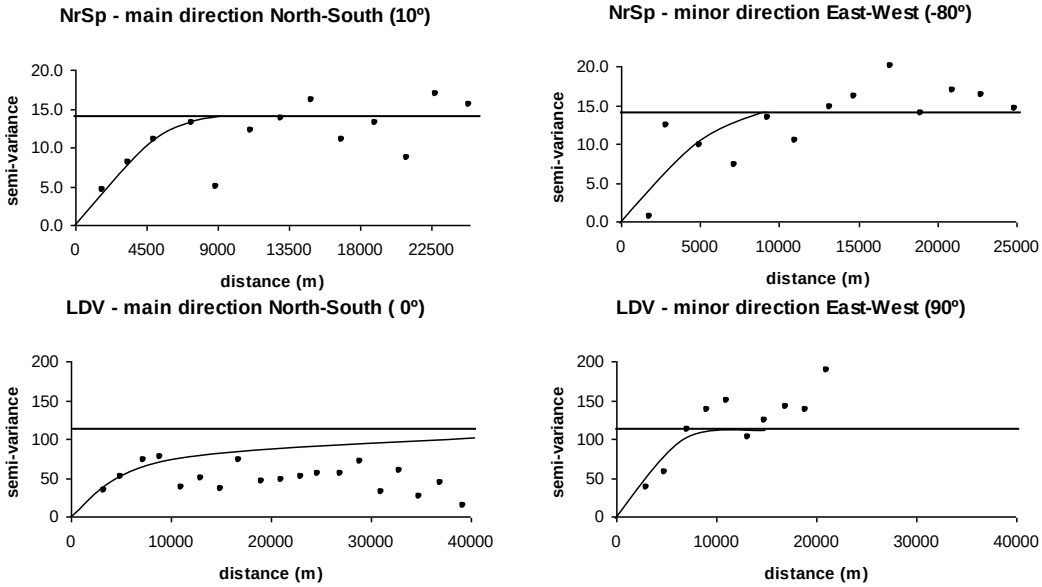

Figure. 2: Plots of the variograms calculated for LDV. Left-hand figure represent the direction of greater spatial continuity (main direction), right-hand figure represent the direction of smaller spatial continuity (minor direction). Semi-variance for each step (distance) is represented by dots, total variance by the horizontal line and fitted model by a curve line. NrSp: number of species; LDV: lichen diversity value.

Analysis at a regional level

The correlations at a regional level between lichen-indicators and the area occupied by land-cover classes (Figure 3) were always negative, showing that the higher the area occupied by "annual cultures", "artificial areas", bare-lands" and "ocean" in the neighbourhood of sampling sites, the smaller the lichen-indicators NrSp and LDV. The bi-plots between those variables suggested that the linear relation was the most appropriated model (Fig. 3). LDV always showed higher significant correlations coefficients (R) than NrSp. The maximum correlation for NrSp was obtained with "artificial areas" (R=-0.44) and for LDV the maximum correlation was obtained with "artificial areas" and "ocean" (R=-0.61). The distance for which a maximum correlation was found varied among land-cover classes and was similar for both indicators: i) for "annual cultures" was 600 m; ii) for "artificial areas" was 1800 m; iii) for "bare-land areas" was 3400 m. However, the maximum correlation for "ocean" was 1800 m for NrSp and more than 6600 m for LDV. In the former case the correlation coefficients were still increasing for the maximum studied distance.

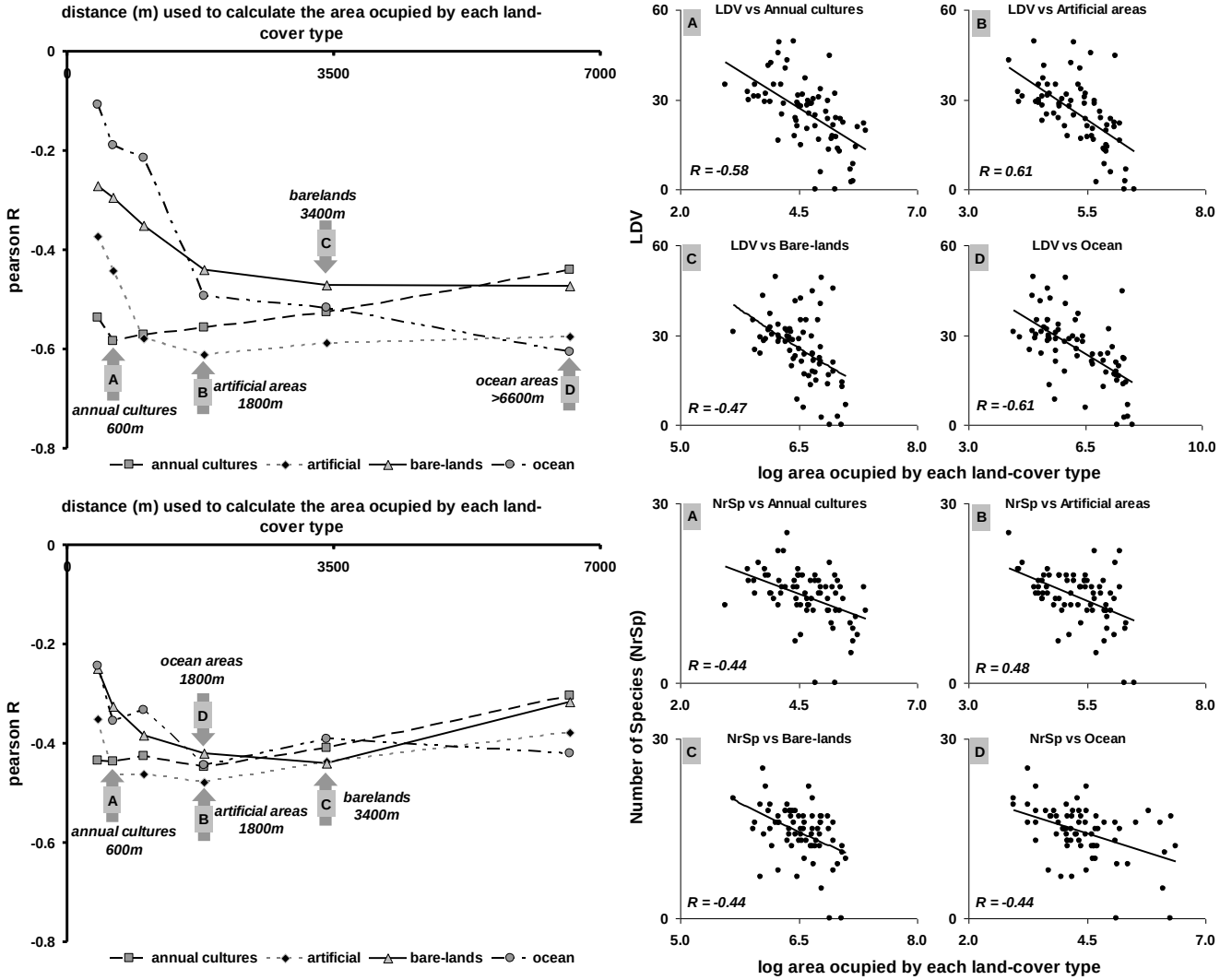

Figure. 3:Results from regional correlation analysis, showing the changes in Pearson R between LDV and NrSp and the area occupied by each land-cover class, when larger areas around sampling sites are considered; considering the distance for which the maximum R value was calculated (indicated by an arrow) the bi-plot is shown.

Analysis at a local level

Identical analysis was carried out at the local level, using sampling sites inside a 10 km circle around each sample site. The calculated correlation coefficients were allocated to the central sampling site (Fig. 1). Since, the neighbourhood distances used for calculating the maximum local correlations changed in space we mapped them (Fig. 4). As occurred in the regional level, local correlations were negative (Fig. 3 and 4). However, while regional correlations had a maximum R of -0.61, local correlations R frequently exceed -0.60 and many exceed -0.80. In general, the sites that showed significant correlations were located in the coastal areas, with the exception of "annual cultures".

LDV decreases with increasing "annual cultures" area mainly in the south of the studied region (Fig. 4). The distance of this land-cover that better explained the changes in the LDV were different: in the lower part of the map 400 m and the upper part of the map 3400 m (Fig. 4). "Bare-lands" areas influenced LDV in a band along the coast with a maximum length of 15 km. The area of this land-cover that better explained the changes in the LDV ranged between 1800-3400 m (Fig. 4). LDV was significantly influenced by increasing "ocean" areas, particularly along the coast. The distance of influence ranged from 3400 m in the northern parts to more than 6600 m in the southern parts of the studied area (Fig. 4). LDV was significantly affected by increasing "artificial areas" up to a distance of 1800 m (Fig. 4). From the observation of the bi-plots between LDV and a land-cover class (Fig. 4) an improved fitting was found, compared to the one obtained at regional level (Fig. 3).

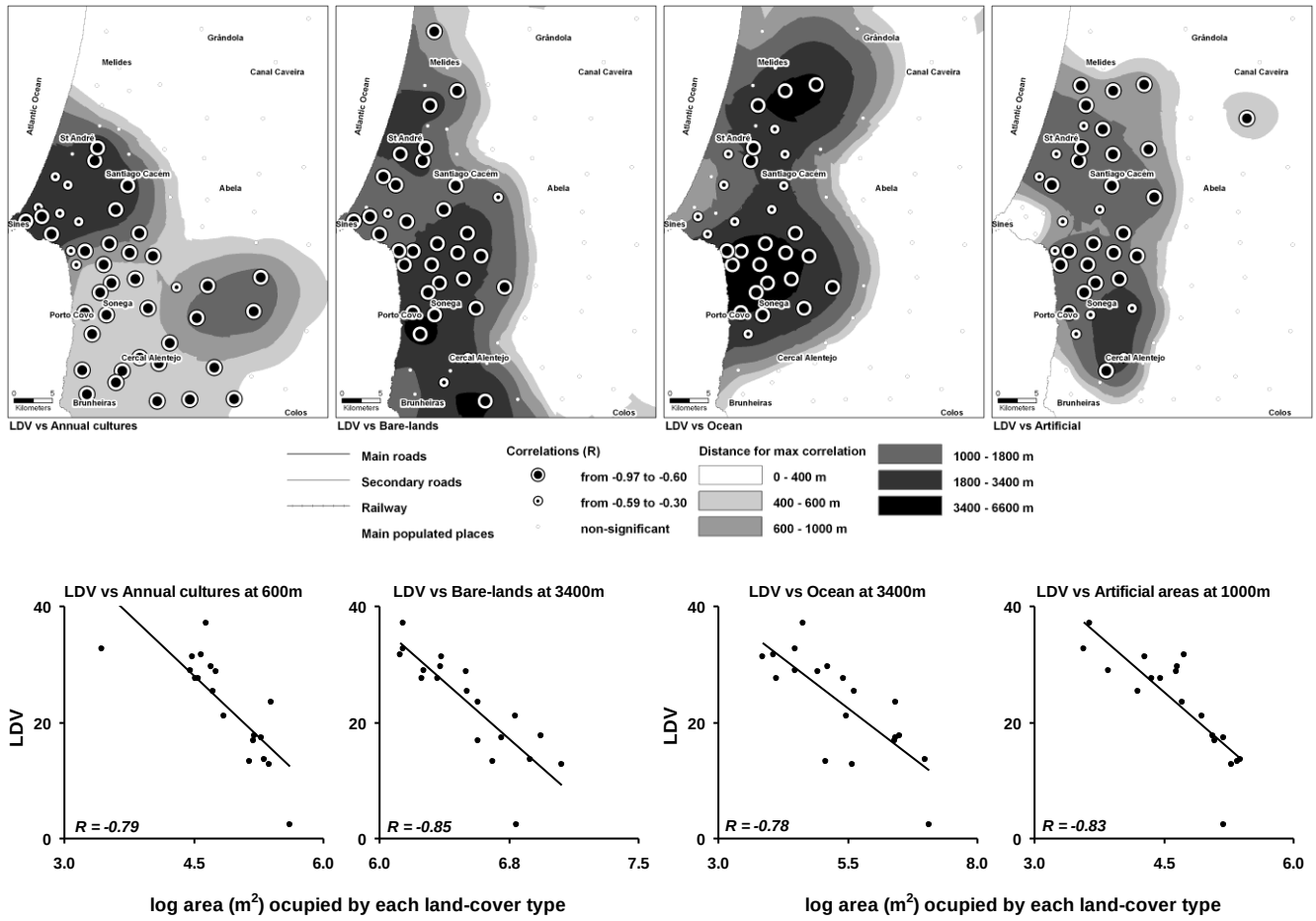

Figure 4: Maps of the local correlation analysis, showing the best correlation between land-cover classes and LDV (circles and dots) and the distance for which the correlation was calculated (grey gradients); an example of a bi-plot is given for one site (the "central site in Fig. 1).

DISCUSSION

The spatial structure of the biodiversity data

The objective of this work was to determine the impact of multiple environmental factors in lichen diversity. For that, in a region with multiple disturbance sources, we first analysed the spatial structure of two commonly used lichen-indicators, NrSp and LDV using geostatistical analysis (Fig. 2). Variograms are not as often used to explore the spatial arrangement of lichen data (Kapusta et al., 2004; Pereira, 1999), as they are used to study and interpolate concentrations of pollutants with biomonitoring data (Augusto et al., 2004; Nunes and Soares, 2005).

Using this geostatistical analysis we found two groups of environmental factors with impact on lichen-indicator LDV, shown by the presence of two structures during the modelling of the data (Table 1). The presence of a strong anisotropy along the North-South direction for this variable (Table 1), indicated that environmental factors were acting mainly in that direction, in accordance with the direction of prevailing winds. The low nugget effect (Table 1) confirmed the reduced influence of site variables (such as tree diameter or land-use intensity) in lichen-indicators, as already observed by Pinho and co-authors (Pinho et al., 2004). This may be related to the fact that in our work, site variables were controlled by always sampling from the same phorophyte and by restricting the range of land-use intensity (Pinho et al., 2004). However, in similar studies a large importance of site variables was found (Hauck, 2005; Kapusta et al., 2004). In the latter work, the obtained variogram presented only one structure and a rather large nugget effect due to sampling sites heterogeneity (Kapusta et al., 2004). Their maximum range regarding NrSp was 3 km, much shorter than the 9 km obtained in this work (Table 1). These results confirmed that our data was adequate for the analysis at local and regional level that was further developed.

How lichen-indicators relates to local and regional land-cover

By looking at the local level analysis we could observe that the impact of most studied land-cover classes was found to be restricted to the first 10 km from the coast, except for "annual cultures" that presented some impact in inland areas (Fig. 4). This result was probably due to the absence of important sources of disturbance in those areas, mainly covered by cork-oak woodlands. Moreover, lichen-indicators did not change much in this inland area being very close to the maximum for the all region.

The local level of analysis was more adequate to interpret the influence of the studied land-cover classes, since higher correlation coefficient values were found with local correlation analysis (Fig. 4) than with regional ones (Fig. 3). The importance of using the land-cover information for explaining the changes in lichens was highlighted in this study by the high correlation coefficients found (Fig 4). The existence of such correlations can be explained by the permanent nature of land-cover, that is, unlike pollutants concentration, not prone to change quickly and dramatically over time.

The distance for which a maximum correlation coefficient was calculated differed among land-cover classes and with the spatial location (Figs. 3 and 4). "Annual cultures" showed to influence LDV and NrSp at the shortest distance, 600 m (Fig 3), which can be related to the presence of grain cultures. These agriculture activities are related with nitrogen rich emissions, particularly NH_3 (Bleeker and Erisman, 1998). Since these agriculture fields are unprotected (without vegetation cover) during at least part of the year, dust rich in particles/pollutants, such as NH_3, might be dispersed from this areas. Dust is considered one of the main causes for the rise in bark pH of *Quercus* trees and by its turn one of the main causes for changes in lichen communities (Loppi and DeDominicis, 1996; Loppi et al., 1997). On the other hand, "bare-lands" areas showed one of the highest distances of influence on lichen-indicators, 3400 m (Fig. 3). This land-cover class accounts for quarries

and other uncovered soil areas originating large amounts of dust that can deplete lichen diversity (Loppi and Pirintsos, 2000; Spencer, 2001).

The range of distance of impact of "bare-lands" is in accordance with other studies performed in the South of Portugal that evaluated the impact of a Cu-mine dust in lichen diversity (Branquinho et al., 1999). The greater distances of influence found in the South of the studied region may be related to the dust dispersion from Sines industrial area, that includes a large quarry but also many areas without vegetation, such as open grasslands.

The influence of artificial areas accounted for the presence of urban and industrial areas and roads, and is probably related to the presence of a large mixture of pollutants, where most of them have a negative effect in a buffer of 1800 m on several lichens species, especially in the non-nitrophytic species (Jovan and McCune, 2005; Loppi et al., 2002; Tretiach and Ganis, 1999) (Fig 3). The presence of "ocean", a natural environmental factor, may account for a large number of factors simultaneously influencing lichens, such as salt spray, altitude and humidity. These factors showed to have the largest regional distance of influence on lichen-indicators, ranging from 1800 m for NrSp to more than 6600 m for LDV (Fig 3). Previous studies in the region showed that most salt-spray is deposited in the first 3 km from the coast (Figueira et al., 1999). However, under specific climatic conditions (strong winds) it may be dispersed much further inland. This difference in the range of distances of influence on lichens might me associated with the size of particles that are dispersed by the different sources. Land-cover class "artificial areas" and "bare-land" areas might disperse dust particles and aerosols, with sizes ranging from very small 1 µm (Morawska et al., 1999) to very large particles, 50-70 µm (McTainsh et al., 1997). These larger particles could derive, for example, from soil re-suspension. On average particle size of "artificial" and "bare-land" areas might be greater than the particle/aerosol size of sea-salt, that ranges from 0.04–0.1 µm (Morawska et al., 1999; Piazzola and Despiau, 1997). This difference in particle size could explain the larger distance of influence that "ocean" areas presented in comparison with "artificial" and "bare-land" areas.

Comparing the lichen-diversity indicators NrSp and LDV

Both lichen-indicators significantly decreased with increasing influence of the studied land-cover classes (Figs. 3). LDV seems more adequate to monitor the influence of land-cover, at least at a regional level, since showed higher spatial continuity (Figs. 2 and 3). The observed difference between NrSp and LDV sensitivity could be explained by: i) the fact that for some environmental factors only lichen frequency or abundance area affected, for example because there was a decrease in suitable areas for growth and colonization, and ii) the fact that, only if environmental conditions become lethal, species can disappear, leading to a reduction in species richness.

Crustose species were not evaluated in the study, although they may account for a significant part of species richness. Nevertheless, several authors (Bergamini et al., 2005) observed that crustose and macrolichen assemblages respond similarly to land-use intensity gradients, so we expect that both groups respond in a similar way to most land-cover changes.

Importance of the spatial framework when designing and interpreting biomonitoring data

In this work we showed that, space, besides variables mapping, and the establishment of a spatial framework, is of crucial importance and strongly contributes to a more clear interpretation of biomonitoring data. The design of such a framework may be considered in two ways for biomonitoring purposes. First when designing a monitoring network, we must consider that the environmental variables have a specific distance of influence on the biomonitors. If this distance is not taken into account an inadequate sampling grid might be designed, in agreement with the results of other authors (Ferretti et al., 2004). For example, in

our study site, using a 4000 m grid for monitoring the influence of artificial areas with LDV is not an adequate, since this land-cover type showed a distance of influence of less than 2000 m (Fig. 3). This occurred when a mapping of the impact of agricultural areas using nitrophytic species was performed with a grid of 6 km (Ruisi et al., 2005). Secondly, when interpreting biomonitoring results in an area with multiple environmental variables, it must be taken into account that different levels of analysis are required to fully understand the results, not only because environmental variables may show different distances of influence, but also because they may be working in different areas of the study region.

Using this method we were able to spatially outline the area of influence of each environmental factor on the lichens (Fig. 4). The results found in this study, are in accordance with results found by other authors (Giordani, 2006; Will-Wolf et al., 2006) that observed the relative importance of environmental factors changes accordingly to the level at which the analysis was performed (from plot to regional scale).

CONCLUSIONS

By determining the impact of neighbourhood land-cover classes in epiphytic lichens richness and abundance we could confirm our hypothesis that lichen diversity is influenced by different environmental factors acting in the same territory and that those environmental factors have different distances of impact on lichens. The analysis of the relation between land-cover classes and lichen-indicators at regional and local level as allowed us to confirm the hypothesis that most studied environmental factors act at the local spatial scale, what could be related to the main particle/pollutants emitted by land-cover class.

This work highlighted the importance of a multiple spatial scale of analysis to deeply interpret the relation between lichen diversity and the underling environmental factors.

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2.2 | Modelling ecology of lichens communities based on photobiont type in relation to potential solar radiation and neighbourhood land-use

ABSTRACT

The objective of this work was to model the ecological response of two lichen groups to key environmental variables, according to the photobiont type, green-alga or cyanobacteria. For that we have sampled epiphytic lichen diversity and abundance in a Mediterranean *Quercus faginea* subsp *broteroi* forest, using a standard method. Lichen functional diversity was not related to forest structure or to short-term indicators of microclimate. However, lichen functional-diversity was highly significantly related to potential solar radiation, an integrated measure of long-term microclimate conditions (light, temperature and humidity). Lichen communities responded to the environmental factors according to photobiont type: green-algae lichens were positively related to potential solar radiation where cyanolichens were negatively related. Moreover, cyanolichens responded negatively to the proximity of neighbouring artificial areas. Lichen communities can be used to: (i.) model long-term microclimatic conditions with high spatial resolution and (ii.) model the disturbance caused by neighbourhood areas, even in natural parks with low-intensity human activities, for conservation purposes.

INTRODUCTION

The diversity and abundance of lichens living in forests surrounded by a human-altered landscape can be influenced by natural and non-natural factors. To model the ecology of those lichen communities in response to key environmental factors, we considered the simultaneous influence of anthropogenic disturbance and microclimate. To help disentangle the effects of both factors we used lichens traits that are functionally related to those factors, namely the lichen photobiont type: green-algae or/and cyanobacteria.

Lichens are one of the most environmentally sensitive groups of organism and have been successfully used not only as monitors of pollution (Geiser and Neitlich, 2007; Giordani et al., 2002; Pinho et al., 2004; Pinho et al., 2008), but also as biomonitors of more complex environmental changes such as habitat fragmentation, habitat stability and influence of forest management (Coxson and Stevenson, 2007; Edman et al., 2008; Nascimbene et al., 2007; Ranius et al., 2008). Although most studies deal with moderate to high disturbance levels, we also need to quantify the impact of low-intensity disturbance in lichen communities. This information is important for policies aiming at Nature conservation, especially in regions where important natural areas are embedded in a landscape mosaic shaped by low-intensity human activities, as in the Mediterranean basin (Blondel and Aronson, 1999). Lichen photobionts are important to consider when dealing with the effect of disturbance. Cyanolichens, for example, were shown to be very sensitive to air pollution, having disappeared from disturbed areas throughout Europe, in particular those subjected to acid rain (Richardson and Cameron, 2004). In California, nitrophytic lichens communities were associated to areas with more agriculture while cyanolichens were nearly absent from those areas (Jovan and McCune, 2004).

It is well known that microclimate influences the distribution, abundance and species richness of lichens. Cyanolichens are known to differ in water requirements as compared to green-algae ones. Cyanolichens require liquid water to start photosynthesis (Green et al., 2002; Lange et al., 1986), and upon rehydration take longer time to achieve maximum photosynthetic rate (Palmqvist, 2000). These results imply that the ecological performance of cyanolichens should be sustained

by longer hydration times, which makes them usually associated to moister and shadier habitats (Maguas et al., 1997). However, there is a surprisingly scarce number of publications linking the known physiological response of species to the community distribution in the field (Jovan and McCune, 2004) and specially to the quantification and modeling of that relations. Water availability for lichens might be influenced by microclimate conditions: light, temperature, and humidity. For epiphytic lichens, forest structure and density are important because they can influence lichen diversity (Caceres et al., 2007; Juriado et al., 2009; Rolstad et al., 2001).

The objective of this work was to model the ecological responses of lichens with two different photobiont types, green-algae lichens and cyanobacteria, to key environmental variables. We sampled epiphytic lichen diversity in a large forest located in a patchy landscape. We also measured an array of potential factors that could drive the lichen distribution pattern: (i) a short-term indicator of microclimate using sensors measurements; (ii) a long-term estimator of microclimate by calculating potential solar radiation; (iii) the forest structure; (iv) the forest NDVI (normalized difference vegetation index) from satellite imagery; and (v) the area occupied by artificial areas in the neighbourhood of the sampling sites, as sources of disturbance.

Modelling key environmental variables that drive lichen communities should support and even develop their power as bioindicators of global change. This understanding should also contribute to better and more integrated conservation strategies of forests, working toward the objectives of the United Nations Convention on Biological Diversity, accorded in 2002, targeting to halt biodiversity loss by 2010 (SCBD, 2006).

METHODS

Study area and sampling design

The study area is located in SW Portugal in a valley of calcareous mountains (max. 678 m) within the Natural Park of Serra D'Aire e Candeeiros. Its Mediterranean climate (Blondel and Aronson, 1999; Thompson, 2005) has a strong Atlantic influence, being located in the thermomediterranean bioclimatic belt (Rivas-Martinez and Rivas-Saenz, 2009). It has dry hot summers and rainy mild winters. Average temperature is 17.5º and average annual precipitation 1400 mm (averages from 1931 to 1960, (IA, 2006). The forest studied is one of the largest *Quercus faginea* subsp. *broteroi* patches (8.46 ha) in the area, ranging from 270-315 m in elevation. The tree layer is dominated by *Quercus faginea* (more than 50% frequency).

The forest patch is located in a Natural Park and is a remnant of fragmentation by human activities. Nowadays the patch has well-defined edges. Adjoining land has natural shrublands (maquis), olive groves and artificial areas (including urban, pasture and agriculture; Fig. 1). Human occupation is of low density, which is revealed by the low-intensity management of olives and agriculture, and little urbanization. The patch lies in a large valley, with a small mountainous area located to the south (Fig. 1). Although human activities surrounding the forest are diverse, no human activities, including logging or cattle access, has been detected in the interior. The forest is fenced on most edges and no trails exist in the interior of the patch.

The patch limit was drawn using aerial photography (year 2005) and sampling sites were positioned using GIS. The first sampling site placed was the geometric centroid, the site that is furthest located from the margin. Afterwards, 27 sampling sites were randomly located within the patch using Random Point Generation tool of Hawth's Analysis Tools (Beyer, 2004) with the following constrains: distance between sites greater than 30 m, in order to be comparable with satellite data resolution (see below) and distance to the margin greater than 10 m (Fig. 1), to avoid direct edge effects.

Lichen diversity sampling and lichen-variables

At each site we sampled epiphytic lichens diversity on the four trees nearest to the GPS location subject

to the following criteria: belonging to *Quercus faginea*, between 18 and 50 cm diameter at breast height, estimated deviation from vertical smaller than 20º, without lateral branches at sampling height and without visible disease. All sampled trees were selected within 10 m of the GPS point. Tree selection criteria were important to minimize the influence of other environmental variables and tree characteristics that were not the focus of this study (Pinho et al., 2004). At each tree the European Protocol (Asta et al., 2002) was applied: a sampling grid with five 10 × 10 cm squares, was placed on each of the trunk's main orientations; all lichen species within the grid were identified, and the number of occurrences was noted as the species frequency.

From this data, we calculated Lichen Diversity Value (LDV), a measure of abundance, as in (Asta et al., 2002). This variable was calculated dividing lichens by functional-groups regarding main type of photobiont (green-algae (including *Trentepohlia*) or cyanobacteria), and dividing species accordingly to growth form: crustose (including leprose), foliose and fruticose.

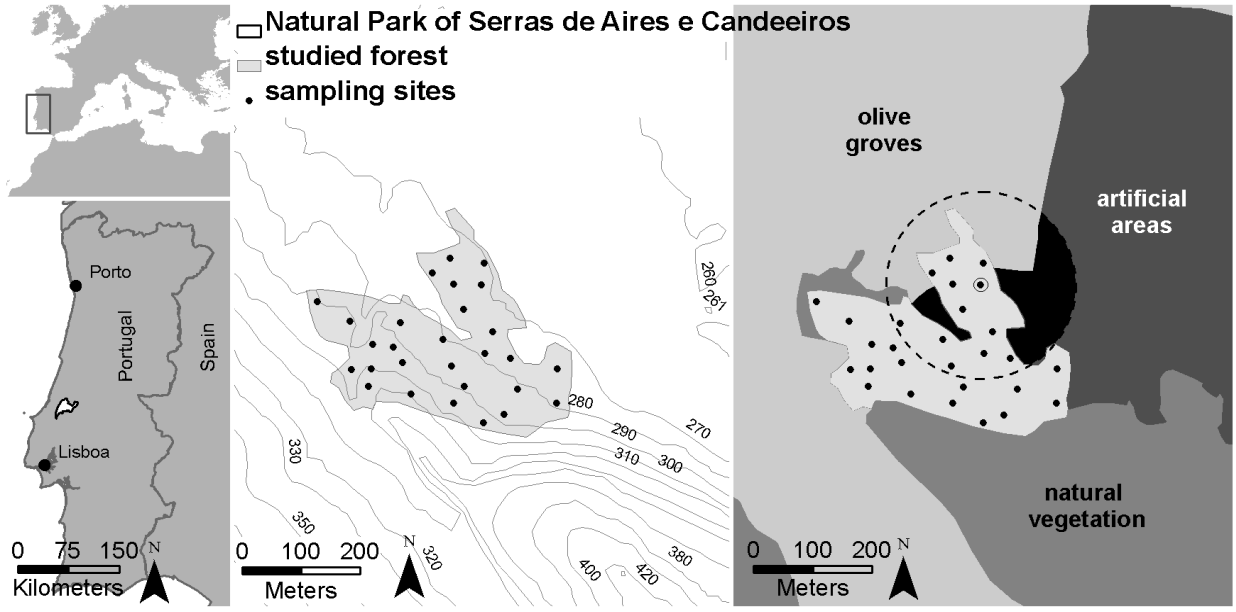

Figure. 1: General location of the study area, showing the Natural Park of Serra D'Aire e Candeeiros (left), location of the studied patch and nearby mountain (centre, values are elevations in meters) and characterization of neighbouring land-cover (right). An example for the calculation of land-cover is given for one sampling site (highlighted dot) and for one distance of influence of the several tested, 160 m (dotted circle). The darkest area is the area occupied by artificial land-cover type considering a neighbourhood of 160 m for the former example site.

Environmental variables

For each sampling site we calculated potential incoming potential solar radiation, using a local digital terrain model (DTM) with 1 m resolution, utilizing Solar Radiation ArcGis tool. This tool calculates the amount of energy potentially arriving to a surface on the ground (direct + diffuse), considering the influence of the site and nearby topography. Values were considered for each month and for a complete year (the sum of the 12 months for 2007). From these values only the one that resulted in the highest correlation coefficient is shown in the results, although significant correlations were found with most of them (data not shown).

Each sampling site was also characterized accordingly to the area occupied by urban and agriculture areas in the neighbourhood of the patch (i.e. outside the patch), considering several distances from the sampling sites (40, 80, 160, 320, 500 m) as in (Pinho et al., 2008) example calculation in Fig. 1). Agriculture and urban areas were considered

together as artificial areas because, in a first approach, their separate analysis gave very similar results regarding the influence on lichens. We tested different distances for calculating the land-use variables, but only shown in the results the one that resulted in the highest correlation coefficient.

A number of other environmental variables were also calculated for each sampling site. These included: (i.) short-term indicators of microclimate, measuring temperature and relative humidity using microclimate sensors during 1 month, May; (ii.) forest structure, determined using field assessment of frequency, density and cover of trees and (iii.) NDVI using LandSat satellite imagery (30 m resolution).

Data analysis and software

Simple linear regressions were performed between lichen variables (green-algae lichens LDV and cyanolichens LDV) and each environmental variables. Potential Solar Radiation was log transformed, improving the distribution of the regressions residuals, which were checked visually in biplots for all regressions. For all analyses, the number of samples was 27 (26 for solar radiation of December) after omitting outliers (more than 4 times the standard deviation).

All GIS operations were performed in ArcGis (ESRI, 2008). Regression were done in Statistica (Statsoft, 2008), data management and graphical outputs in EXCEL (Microsoft, 2007).

RESULTS

A total of 61 lichen species were found, most of them foliose (62%). Foliose species accounted for 74% of LDV on average (Fig. 2). Most lichens species had green-algae as the main photobiont (66%) and these lichens accounted for 70% of the total LDV (Fig. 2). Number of species containing green-algae ranged from 3 to 26 and their LDV from 0,8 to 51,3. Cyanolichens ranged from 0 to 10 species per site and LDV from 0,0 to 19,3. No correlation was found between the LDV of green-algae lichens and that of cyanolichens (Fig. 2).

No correlations were found between lichen-functional diversity variables and short-term indicators of microclimate measured by sensors for one month, May 2007 (data not shown). No correlations were found either with forest structure variables, including frequency, density and cover of trees, or to NDVI (data not shown). The LDV of green-algae lichens was positively related to potential solar radiation calculated for June (Fig. 3). The LDV of cyanolichens was negatively related to neighbouring artificial areas, based on a 160 m neighbourhood, and to potential solar radiation for December, although the somewhat weaker (Fig. 3).

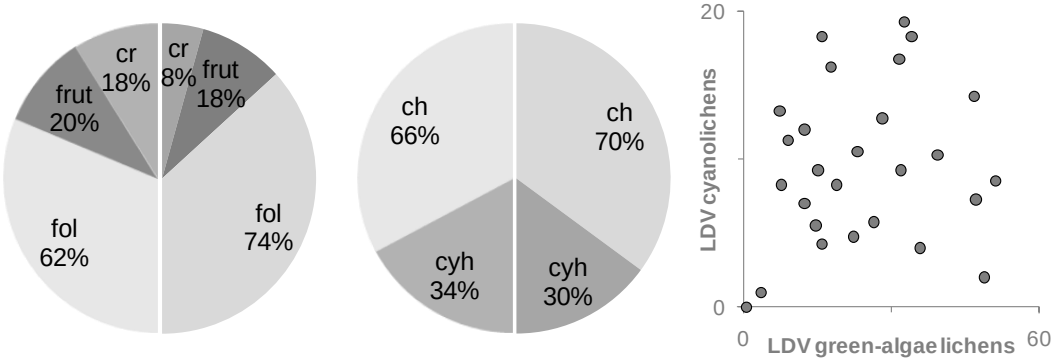

Figure. 2: Relative contribution of lichens divided accordingly to growth form (left) and photobiont (centre). Left portion of the diagrams refers to the species richness the right one to the LDV. fol: foliose, frut: fruticose, cr: crustose; ch: lichens with green-algae, cyh: lichens with cyanobacteria. Right: plot of the LDV of green-algae lichens versus cyanolichens.

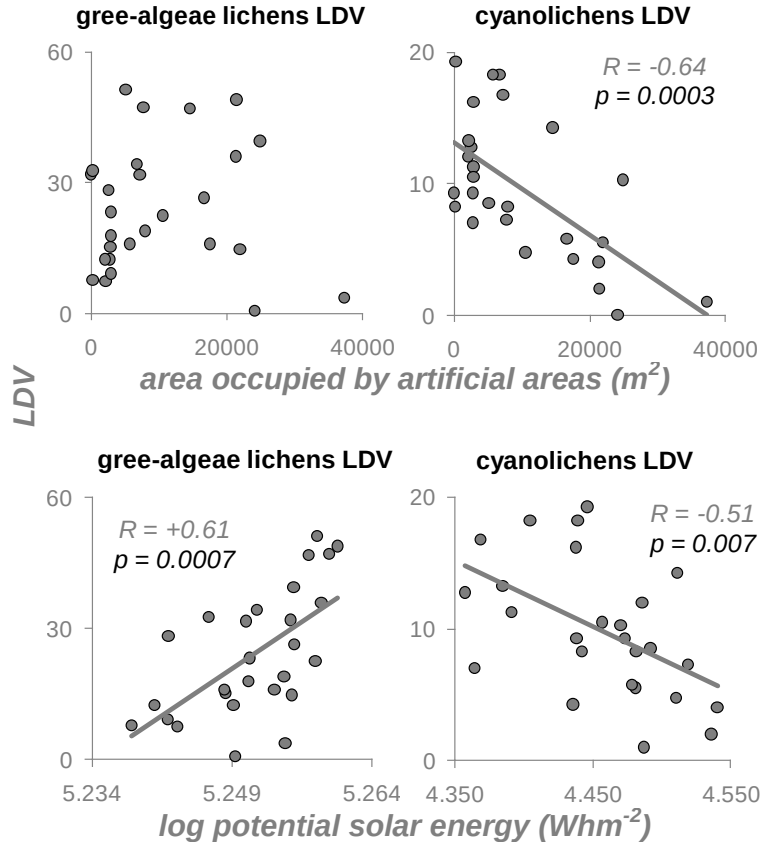

Figure 3: Correlation between lichen functional-diversity variables and neighbouring artificial areas and potential solar radiation. Neighbouring artificial area is the area occupied by agriculture and urbanization and the highest correlation coefficient was obtained considering a neighbourhood of 160 m. Potential solar radiation is a measure of the amount of potential incoming energy and the highest correlation coefficient was obtained using the values for June (for green-algae lichens) and December (for cyanolichens). Pearson correlation coefficients and associated p value are only shown for significant correlations.

DISCUSSION

We found that lichen functional-diversity in the studied forest was related to long-term microclimatic conditions and to neighbouring artificial land-cover, but not to forest structure or to short-term indicators of microclimate. These responses differed by photobiont type. We infer, therefore, that this trait is at least partly responsible for shaping the niches occupied by green-algae lichens and cyanolichen species.

Foliose lichens were the dominant growth form both in number of species and abundance (Fig. 2). According to other authors (Aragon et al., 2006; Fos, 1998), these forests may present a relative higher number of crustose species. Our results could be due to the fact that we sampled only on the main trunk of the tree, where competition with foliose lichens and bryophytes can be higher than on small branches. Moreover the contribution of cyanolichens, mostly foliose species, was very high in this study, representing more than 30% of species richness and abundance, in contrast to 7% contribution of species richness in other studies (Fos, 1998). This high percentage of cyanolichens could be due to the high average annual precipitation levels in this area that is 1400 mm (averages from 1931 to 1960 (IA, 2006).

Key factors influencing lichen communities

We modelled the relationships of photobiont types to key environmental variables. The use of functional groups allowed us to disentangle the influence of the environmental factors, in accordance with several studies (Hilmo and Holien, 2002; Root et al., 2007; Stofer et al., 2006; Wolseley

and AguirreHudson, 1997). Moreover, no correlation was found between cyanolichens and green-algae lichens (Fig. 2) indicating that the environmental factors did not influence lichen functional groups in the same way.

Because microclimate is strongly influenced by the forest structure, we first hypothesized a dependence of lichen communities on forest structure. However, no clear response was identified in relation to several forest parameters, namely frequency, density and cover of trees, or to NDVI (data not shown). Additionally no correlations were found with the short-term indicators of microclimate (data not shown). However, two environmental factors have shown to be significantly related to the distribution of lichen communities: potential solar radiation (a long-term measure of microclimate), and the presence of neighbouring artificial areas (a measure of disturbance) (Fig. 3).

These factors were related to the photobiont of lichens (Fig 3). Potential solar radiation was related to both green-algae lichens and cyanolichens (Fig. 3): green-algae lichens were more abundant in sites with higher potential solar radiation and cyanolichens less abundant in those sites (Fig. 3), although the correlation with cyanolichens was less significant. Potential solar radiation measures the amount of radiation arriving to the canopy, and other similar methods were previously developed for estimating this important ecological factor (McCune, 2007; McCune and Keon, 2002). It is an estimation of long-term macroclimate, areas with more solar radiation should experience higher temperatures, lower humidity and higher light availability (Dyer, 2009). Potential solar radiation has been applied to determining the potential occurrence of invasive species (Pande et al., 2007), community responses to clear-cutting (Astrom et al., 2007) and prediction of plant and lichen communities distribution (Becker and Brandel, 2007; Holt et al., 2009), where it was found to be a good predictor of xeric versus moister sites. The results accord with the known tolerance of green-algae lichens to water-loss, due to their capability of rapidly initiating photosynthesis with only water vapour (Lange et al., 1986). The lower statistical significance of cyanolichens response (Fig. 3) could be due to the action of other more powerful environmental factors. Furthermore, the cyanolichen group included some *Collema* and *Leptogium* species that were shown to be tolerant to more open conditions caused by selective logging (Hedenas and Ericson, 2003; Hedenas and Hedstrom, 2007). These results therefore provide an ecological significance to the known physiological response, and reinforce the use of potential solar radiation as a powerful estimator of long-term macroclimate.

The other major environmental variable, artificial areas, was also related to lichen communities: although green-algae lichens were not related to artificial areas, cyanolichens were strongly so (Fig. 3). The presence of artificial areas, in this case mostly low-intensity agriculture and scattered urbanization, can influence nearby natural areas in different ways, including by direct disturbance (Broadbent et al., 2008; Hamberg et al., 2008; Hobbs, 2001; Kupfer et al., 2006) or by emitting pollution (Gove et al., 2007). In our study area, direct disturbance was unlikely, because no evidence of access to the forest was found and most edges are fenced. Therefore the most likely cause for the impact of artificial areas in cyanolichens is the emission of ammonia and dust from nearby artificial areas. This is in agreement with known effects of ammonia and dust on lichen-functional diversity (Loppi and Pirintsos, 2000; Pinho et al., 2009; Stofer et al., 2006), and to the high sensitivity of cyanolichens to pollution (Richardson and Cameron, 2004). These results regarding the influence of neighbouring artificial areas may have broad applicability, such as monitoring for conservation purposes. Most studies are concerned with the existence or not of suitable nearby habitats for the conservation target species (Hedenas and Ericson, 2008). However, the surrounding matrix must also be considered as a potential disturbance source, even if the neighbouring land-use is of low-intensity. Even low-intensity human pressure might have a large effect on sensitive lichen communities.

Species niches within these communities were linked to different drivers. Green-algae lichens were

related to potential incoming radiation, preferring the most exposed sites, and were independent of the low intensity disturbances associated with neighbouring artificial areas. Green-algal lichen LDV varied between c. 10 (in the most shaded conditions) to c. 50 (in the more exposed ones; Fig. 3). Cyanolichens were related to potential solar radiation but was mainly modelled by the influence of neighbouring artificial areas (Fig. 3). In fact, we could find both low and high values of cyanolichens LDV in areas with high solar radiation. However, when artificial areas are considered, we could find the lowest LDV of cyanolichens in sites with greater artificial influence (i.e. more than 7000 m^2 at a distance of 160 m), and much higher LDV of cyanolichens when the influence of artificial areas decreased.

The most innovative aspects of our results were that: (i.) Lichen-functional groups provided a link between the known physiological response of lichens to their distribution in the field. (ii.) To achieve the objective of halting biodiversity loss by 2010 (SCBD, 2006), especially in natural areas surrounded by human activities, the neighbouring areas must be considered potential disturbance sources, even if the land-use is of low-intensity; these areas must therefore be managed accordingly. (iii.) Quantification of lichen functional-group niches can improve conservation strategies for sensitive habitats by validating the use of lichens as ecological assessment tools; e.g., for mapping the effect of disturbances and long-term microclimate conditions.

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chapter 3

03 | Temporal and spatial patterns of lichens functional groups as ecological indicators of the local effects of global-change drivers within Mediterranean forests

ABSTRACT

There is currently a need to understand the effects of global change drivers in ecosystems, not only at a planetary scale but also locally, where the management actions to ameliorate those effects can be implemented. Ecological indicators can provided a method to map, with high spatial resolution, the local effects of global change drivers. Our objective was to determine the spatial and temporal patterns of lichen functional groups based on the main photobiont-type in response to climate alterations. We considered a climate gradient driven by orography and measured by potential solar radiation, and tested its effects on epiphytic lichens functional groups within a Mediterranean *Quercus faginea* forest. Lichens functional groups were shown to respond to the spatial microclimatic gradients, and that response was depended on the photobiont type: cyanolichens were negatively related to potential solar radiation, in particularly during wintertime, while green-algae lichens were positively related, particularly during summer season. Thus lichens functional groups based on photobiont type could be used as ecological indicators to spatial and temporally modeling the local effects of climate change, both seasonally and spatially.

INTRODUCTION

The global change phenomenon includes a series of alterations in planetary systems (IGBP, 2007; Steffen et al., 2004), of which three are an increasing concern: the rate of climate change, of biodiversity loss and of interference with the nitrogen cycle (Rockstrom et al., 2009). These drivers were considered not only to have crossed a safety threshold beyond which human activities may no longer be sustainable (Rockstrom et al., 2009) but were also considered the drivers with increasing effects on all Earth biomes (MEA, 2005a). This work was focused on the effects of spatial climate alterations. The predictions made on climate changes vary according to location. For Mediterranean area projections include an increase in drought conditions, due to higher temperature and reduced precipitation (IPCC, 2007). The effects of climate changes are studied using long-time climatic data-series (IPCC, 2007). However, when searching for indicators of climate alterations in a short period one may look at a climate gradient in space, because multiples points in space contain more information that one point measured multiple times (Dakos et al., 2010; Donangelo et al., 2010; Scheffer et al., 2009).

Although the drivers of climate change work at a planetary scale, their influence on ecosystems must be assessed at a regional or local spatial scale (Hansen et al., 2010; Russell et al., 2009). Knowing the local effects on ecosystems may allow management actions aimed at sustaining ecosystems biodiversity and functioning and their possibility to provide the services needed for humanity (MEA, 2005b). The use of ecological indicators, namely lichens, could provide a high spatial resolution method for mapping the spatial effects of climate alterations. Lichens are organisms that result from the symbiosis between a fungi and one or more photosynthetic partner, a green-algae or a cyanobacteria (Honegger, 1991). Lichens have particular physiological characteristics, such as the absence of a cuticle or roots, which makes them good ecological indicators (Honegger, 1991). Lichens are frequently used to monitor environmental changes (Cape et al., 2009; Pinho et al., 2009) and have been suggested as potential ecological indicators for monitoring the effects of climate changes due to their sensitivity to

microclimate variables (Aptroot and van Herk, 2007; Giordani and Incerti, 2008; van Herk et al., 2002). However, the use of lichens as ecological indicators for the effects of climate alterations is currently having two main limitations.

A first concern is related to the type of measures of lichen communities. A common measure of lichen used as ecological indicator is total diversity, but this lichen variable may group species with opposing responses to environmental factors, as in the case of eutrophication (Pinho et al., 2009; Van Dobben and Ter Braak, 1998). An alternative to total diversity is to use of functional traits, especially those that can be mechanistically related to the effects of climate alterations. One of those traits is lichen main photobiont type, which can be green-algae or cyanobacteria. Cyanolichens require liquid water to start photosynthesis (Green et al., 2002; Lange et al., 1986) and that upon rehydration take longer to reach maximum photosynthetic rate (Palmqvist, 2000). Unlike cyanolichens, green-algae ones can initiate photosynthesis with water vapor (Green et al., 2002; Lange et al., 1986) and once active reach faster the maximum photosynthetic rate (Palmqvist, 2000). Therefore, the ecological performance of cyanolichens should be sustained by longer hydration times as compared to green-algae ones, associating cyanolichens to more moist and shadier habitats (Maguas et al., 1997). Thus, the use of lichens functional groups based on the main photobiont type seems an attractive approach to study the local effects of a spatial gradient of climate alterations.

The second concern is the lack of suitable characterization of lichen-variables regarding their spatial and temporal patterns in response to environmental changes. The creation of spatially explicit mapping of the effect of any environmental change is crucial. This mapping allows not only the location of the effects (Pinho et al., 2008a) but also allow to provide adequate management responses to where they are most needed (Metzger and Schroter, 2006). The seasonal influence in lichens response to climate variation is not clear. As any other photo-autotrophic organism, lichens need water and light to grow, but, because lichens are poikilohydric organisms, the hydration time regulates the period during which metabolic activity is possible and growth may occur (Lange, 2003). Given the highly diverse conditions where lichens may present metabolic activity necessary for growth (Caldiz, 2004; Hale, 1973) it is important to known the critical periods associated to environmental variables, which will lead to changes in metabolic activity and consequently to growth and distribution.

The objective of this work was to determine the spatial and temporal patterns of lichens functional groups based on the main photobiont type, in response to a spatial gradient of climate alterations. For that we used a spatial climate gradient driven by orography, and tested its effects on epiphytic lichens within a Mediterranean forest. Ultimately, this could allow us to select early-warning indicators for the effects of climate change, because ecosystems responses to environmental changes are not necessarily linear and a small change in environmental conditions may originate a large shift in the ecosystem (deMenocal et al., 2000).

MATERIALS AND METHODS

Study-area and sampling design

The study area was located in SW Portugal in a calcareous mountain (max. 678m) included in the Natural Park of Serra d'Aire e Candeeiros (PNSAC). It belongs to the thermomediterranean climatic belt (Rivas-Martinez and Rivas-Saenz, 2009) presenting a Mediterranean climate, characterized by dry hot summers and rainy mild winters (Blondel and Aronson, 1999; Thompson, 2005). The average temperature is 14.8ºC and average annual precipitation 844 mm (averages from 1960 to 1990 at Monte Real). The study site is a *Quercus faginea Lam.* forest (8.46ha).

The studied forest presents clear borders and a neighboring land-use of natural scrubland vegetation (maquis), other forest areas, olive groves and artificial areas mostly composed of traditional agriculture activities. For the smallest administrative

division, civil-parish, the population density is 26 habitants km^{-2} (INE, 2001). The interior of the forest was devoid of any recent human activity, which was confirmed by the absence of trails inside the forest. This is important because forestry activities are known to influence biodiversity, including lichens (Broadbent et al., 2008; Hamberg et al., 2008; Hobbs, 2001; Kupfer et al., 2006), ensuring in this way that the land-use intensity of the sampling sites was homogeneous. Within the forest twenty eight sampling sites were distributed (Fig. 1, centre) using Random Point Generation tool of Hawth's Analysis Tools (Beyer, 2004) as described elsewhere (Pinho et al., 2011). In each sampling site we characterized the lichen communities and the long-term microclimate.

Environmental and lichen-diversity variables

A climatic gradient was established within the studied forest, by looking at the spatial variation of Potential Solar Radiation (PSR). PSR was determined for each sampling site using a digital terrain model with 1m resolution, by running the Solar Radiation ArcGis tool (Fig. 1, left). This tool considers the influence of the site and nearby topography to calculate the amount of energy, including the direct but also diffuse energy that is potentially arriving to a flat surface located on the ground. It does not however take into account the presence of clouds or other atmospheric characteristics. Nevertheless, the cloud conditions that occur in a small area such as the studied forest are probably very similar for the different sampling sites. The values of PSR were calculated for each month (Fig. 1, right). Because PSR is the approximate amount of energy reaching the canopy every year it was inferred as the long-term microclimate at each sampling site. This is was possible since forest structure, very homogeneous in the studied area, was found not to be related to lichens (Pinho et al., 2011).

Epiphytic lichens were sampled in *Q. faginea* as described in Pinho et al. (2011) using the European Protocol (Asta et al., 2002). In the four trees nearest to the GPS location of each site, a sampling grid with five 10*10cm squares was placed in each main orientations of the trunk. All lichen species within the grid were identified, and the number of occurrences was noted as the species frequency. From this data, we calculated a lichen diversity value, LDV (Asta et al., 2002), a measure of lichens abundance. LDV was calculated for two lichen functional groups, based in the main photobiont type (Nimis and Martellos, 2008): green-algae (LDVch) or cyanobacteria (LDVcyh). Since we considered the main photobiont type, tripartite lichens such as *Lobaria pulmonaria* and *Lobaria amplissima* were considered in the green-algae group.

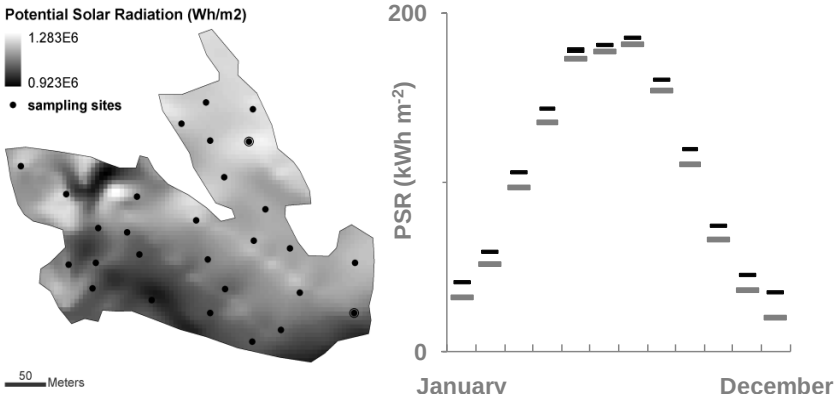

Figure 1: **left**: potencial solar radiation (PSR) calculated from a digital terrain model, showing values in Watts hour m^{-2} for a complete year, 2007; sampling sites are also shown; **right**: montly variation of PSR in two example sampling sites, highlighted on the left figure.

Data analysis and software

We tested how the functional lichen-variables LDVch and LDVcyh responded to a spatial gradient of climate alteration by relating the LDVs to potential solar radiation using simple linear regressions. When necessary the variables were log before the analysis, improving the distribution of the regressions residuals, especially the homogeneity of variance and adequacy of residuals to the normal distribution, which were checked visually in biplots for all regressions.

We determine the temporal characteristics of LDV as indicator of microclimate conditions, by calculating a correlation between the LDVs and PSR for each month. The month with the more significant correlation coefficients was considered as the time-of the-year with most influence (Pinho et al., 2008b). We calculate variograms of the LDVs, in order to analyze their spatial characteristics. In the variograms the range corresponds to the distance upon which the variable presents spatial continuity; the ratio nugget/total variance corresponds to the portion of variance at short range without a spatial structure (values closer to 1 indicating larger portion of variance without spatial structure). Finally, for mapping the LDVs as ecological indicators of the effects of spatial climate alterations, a function fitted to each of the empirical variograms was applied to interpolate LDVs within the studied forest using ordinary kriging.

All GIS operations and mapping outputs were performed in ArcGis v. 9.3 (ESRI, 2008) except variogram and kriging that were done in GeoMS (CERENA, 2000). Regressions were done in Statistica (Statsoft, 2008), data management and plots in EXEL (Microsoft, 2007).

RESULTS

The mapping of PSR resulted in a spatial pattern within the studied forest that varied from more exposed areas in the north to less exposed ones in the south (Fig. 1, left), due to the local orography and to the protection of nearby mountains located to the south. We could find very high seasonal variations of PSR, with much higher values calculated for the summer than for the winter (Fig. 1, right).

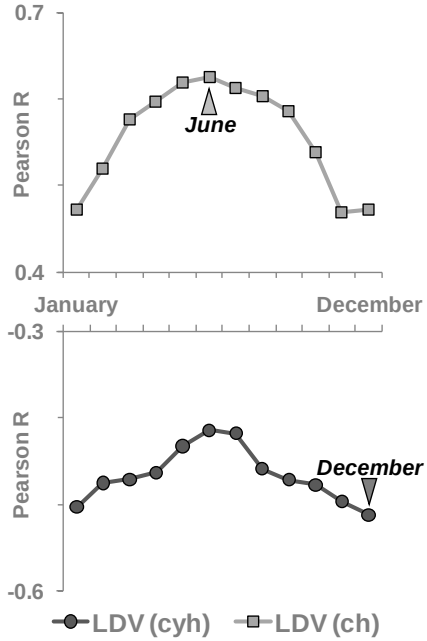

Figure 2: Plot of the Pearson R between Potential Solar Radiation (calculated for each month) and the LDV of green-algae lichens (above) and LDV of cyanolichens (bellow). The arrows show the month for which the correlation coefficient was more significant, June for LDVch (R= +0.62, P=0.0006), December for LDVcyh (R=-0.51, P=0.0075).

Temporal characteristics of the lichen-variables as ecological indicators of microclimate alterations

Based on functional diversity we calculated the values of LDV of green-algae (LDVch) and cyanolichens (LDVcyh) that were then related to PSR calculated for each month and the correlation coefficients (R) obtained were plotted (Fig. 2). Statistical significant correlations ($P<0.05$) were found for most months, although with different strength and signal, which depended on the functional group: the correlation between PSR and LDVch was positive while that of LDVcyh was negative. Moreover, a temporal pattern of correlations coefficients was found (Fig. 2): the season with the more significant correlation was during summer months for LDVch (June, R= +0.62, p=0.0006) while it was the winter months for the LDVcyh (December, R=-0.51, p=0.0075).

The spatial structure of LDVs was analyzed using geostatistics by calculating the variograms of LDVch and LDcyh, showing similar characteristics for the two lichen-variables (Table 1). The spatial continuity, measured by the range for both variables was more than 180m (average distance between all sampling sites was 170m). Additionally, both LDVs presented a strong spatial structure on the short range, indicated by the 30% of the total variance explained by the nugget effect, for both for LDVch and LDVcyh.

Considering their spatial structures (Table 1), the LDVs were interpolated within the studied forest using ordinary kriging (Fig. 3). Although the spatial characteristics of the LDVch and LDVcyh were similar (Table 1), their mapping has shown different spatial patterns. The LDVch was higher in the northern part of the forest and decreased to the south and in the interior, while the LDVcyh was higher in the western and interior areas of the forest.

Table 1: Spatial characteristics of the function fitted to the variogram of LDVs. Range indicates the distance up to which the variables present spatial continuity, the ratio nugget/total variance the proportion of short-range variance without a spatial structure (values closer to 1 indicating no spatial structure) and fitted function the type of function fitted to the experiment variogram.

variable	range (m)	nugget/total variance	fitted function
LDV green-algae lichens	180	0.33	spherical
LDV cyanolichens	200	0.36	spherical

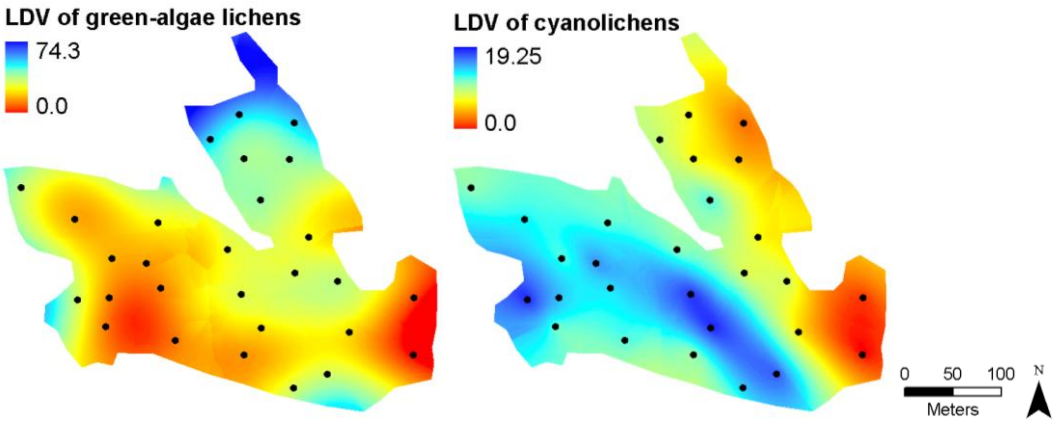

Figure 3: Interpolation of the LDVs of lichens functional groups based on the main photobiont type as ecological indicators of the spatial effects of climate alterations. The LDV of green-algae lichens (left) and LDV of cyanolichens (right) were interpolated using ordinary kriging after variogram analysis (Table 1). Blue colors represent higher values, red colors lower values.

DISCUSSION

The PSR is a measure of the amount of radiation arriving to the forest that was obtained from the orography. We used it as an estimation of long-term microclimate, because areas with higher PSR should experience higher temperatures, lower relative humidity and higher light availability on the long-term (Dyer, 2009). Being an important ecological factor (McCune, 2007; McCune and Keon, 2002) PSR has been frequently used in ecology for estimating the occurrence of invasive species (Pande et al., 2007), community's response to forestry (Astrom et al., 2007) and as predictor of lichens and plant species occurrence (Becker and Brandel, 2007; Holt et al., 2009). Additionally, it could also be used as an estimator for spatial microclimate alterations. Therefore by looking at the relations between PSR and LDVs we evaluated the long-term effect on lichens of a spatial environmental climate gradient, from moister to xeric conditions to be able to test potential ecological indicators of climate change.

PSR was related to lichens functional groups, but the direction and intensity of the relation and also the time-of the-year of most effect depended on a functional trait:, the main photobiont type (Fig. 2). The dissimilar response of LDVch and LDVcyh to PSR was expected, due to the physiological characteristics of each functional group (Honegger, 1991), most probably related to the lower tolerance of cyanolichens to more xeric conditions (water and light) when compared to green-algae ones, in accordance to what was found in previous works (Green et al., 2002; Lange et al., 1986; Maguas et al., 1997). This different tolerance could explain why cyanolichens are more abundant in the shadier areas of the forest and green algae ones are more abundant in the more light exposed areas (Maguas et al., 1995) (Fig. 3).

The lower degree of importance of PSR in explaining the distribution of LDVcyh compared to that of LDVch (Fig. 2) could be due to some heterogeneity of species response within the LDVcyh functional group. Examples of this are *Collema* spp. and *Leptogium* spp., which area cyanolichens but that have some degree of drought tolerance demonstrated in several situations namely after logging (Hedenas and Ericson, 2003; Hedenas and Hedstrom, 2007). Another possible explanation could be the presence of another environmental factor influencing LDVcyh compared to LDVch. Neighboring agriculture areas can disperse atmospheric NH_3 to the nearby forest and negatively affect this functional group (EPER, 2004; Galloway et al., 2003), causing biodiversity loss (Phoenix et al., 2006; Suding et al., 2005) including in lichens (Loppi and Pirintsos, 2000; Pinho et al., 2009; Stofer et al., 2006) specially on the more sensitive to pollution, the cyanolichens (Richardson and Cameron, 2004). Thus, most probably eutrophication could be one environmental factor affecting only the cyanolichens group and reducing the significance of its response to PSR (Pinho et al., 2011).

All studies agree that, besides the requirement for water, the photosynthesis in most green-algae lichens is limited by low temperatures and light (Armstrong, 2006). For example *Lobaria pulmonaria* (main photobiont green-algae), was shown to grow more in Portugal than in Sweden, due to more favorable temperatures and light availabilities (Gaio-Oliveira et al., 2004). Because PSR values of May to July are the highest of the year (Fig. 1, right) and LDVch has shown significant and positive relations to it (Fig. 2) we could conclude that the spatial pattern of this functional groups is determined by more sunnier or xeric environmental conditions, which could be seen both in space (i.e. within the forest, Fig. 3 left) and time (i.e. June, Fig. 3). This might happen because if they are limited by light availability during the rest of the year. Naturally, for growing during the summer season water must be available. Green-algae lichens are known to require only water vapor to start photosynthesis (Lange et al., 1986) and therefore it feasible that they acquire water even during the Mediterranean summer (typically with very low precipitation), from the morning condensation and from the rare rain events.

Unlike green-algae lichens the cyanolichens require liquid water to start photosynthesis (Lange et al.,

1986) and therefore are associated to moister areas (Maguas et al., 1997). This characteristic explains their negative relation to PSR (Fig. 2, bellow) and why these lichens are more abundant in the shadier areas of the forest (Fig. 3). Interestingly, the correlation between LDVcyh and PSR was more significant during the winter months, especially December (Fig. 2). The *Quercus faginea* is marcescent tree, keeping the dead leaves during the autumn and most of the winter (Bingre and Damasceno, 2007). For this reason the time-of-the-year with less leaves goes from late December until early spring, when the new leaves appear. During this critical period, with colder temperatures and stronger light intensity, there are conditions for the occurrence of photo-inhibition (Demmig-Adams and Adams, 2000; Demmig-Adams et al., 1990), a reduction of photosynthetic activity due to excess light. The differences between functional groups response are according to the observed differences in the pigments composition of both PSI and PSII, suggesting basic differences in what concerns carotenoids photo-protection (Demmig-Adams et al., 1990)

Lichen functional groups based on the main photobiont type, a green-algae (LDVch) or cyanobacteria (LDVcyh), were shown to be good candidates for ecological indicators of microclimate alterations. Besides mapping with high resolution the effects of climate alterations, lichen functional groups could also be use as an early-warning system in order to detect the first effects of climate change in ecosystems before sudden shifts occur for other components not so sensitive (deMenocal et al., 2000).

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chapter 4

04 | Effects of low intensity land-use on epiphytic lichen communities under Mediterranean climate

ABSTRACT

The effects of land-use in species and communities are usually studied by considering high-intensity land-use gradients. However, in many European areas, the traditional land-use regime is of low-intensity, associating light forestry practices and extensive livestock pasture. This low-intensity land-use is associated with High Nature Value Farmlands, which occupy more than 50% of total farmland area in Mediterranean areas. The objective of this work was to determine the effect of a traditional low-intensity land-use in epiphytic lichen communities in Mediterranean cork-oak woodlands. We intend to investigate the ecological value of lichen community as an early indicator of the impact of land-use changes. For that, lichens were sampled in a gradient of low-intensity land-use that included forestry and pasture activities. Within lichen diversity we considered: i) total species richness; ii) total species abundance (LDV); and iii.) richness and abundance of functional groups, dividing species according to eutrophication-tolerance. The results have shown an increase in total species richness with a very low increase of land-use intensity, which was shown for first time in lichens. That was a result based on the increase of the number of tolerant species versus maintenance in the richness of the sensitive ones. These results fit with the intermediate disturbance hypothesis that predicts higher diversity under an intermediate disturbance, due to the promotion of the coexistence of species with contrasting ecological requirements. Traditionally managed cork-oak woodland with low-intensity land-use can thus support high diversity of lichen species. Since lichens diversity was shown to respond to low levels of land-use intensity, we confirmed the importance of using ecological-indicators based on functional-traits for monitoring changes in ecosystems and also the possibility of using lichens as early-indicators of the impacts of low intensity land-use.

INTRODUCTION

Traditional agro-ecosystems are characterized by low nutrient input and relative low output per area (Plieninger et al., 2006). Cork-oak woodlands are good examples of such low input-low output system. As in many other European areas, these traditionally managed systems are species rich landscapes of considerable conservation value and which are now the target of conservation policies (Plieninger et al., 2006). Accordingly, these areas were classified as "High Nature Value Farmland" (Paracchini et al., 2008). The percentage of total farmland area that could be classified as High Nature Value Farmland reaches 32% in overall Europe, although a difference exists between Mediterranean countries (52%) and non-Mediterranean ones (27%) (Paracchini et al., 2008). Regarding the traditional agro-ecosystems, one important question left open is how low-intensity management activities influence the biodiversity and ecosystem functioning. The potential use of the lichen community as early indicators of the impact of changes in land-use and its intensity is therefore an important issue to consider within both ecology and conservation purposes.

Lichens are unique organisms which consist of a fungal partner, as well as, one or two photosynthetic partners (green algae and/or cyanobacteria). Due to their physiological characteristics (Honegger, 2009) they are sensitive to a wide range of environmental impacts. In the last decades, numerous studies successfully used epiphytic lichen diversity as an environmental indicator, especially of the impact of air pollution (Giordani, 2007; Giordani et al., 2002; Nimis et al., 1991). In woodlands, far from urban or industrial areas, management activities such as forestry (Coxson et al., 2003; Dettki and Esseen, 1998, 2003; Hedenas and Ericson, 2003), agriculture and livestock grazing (Ruoss, 1999; van Herk, 1999; van Herk, 2001) have been recognized as the most

important driving forces for epiphytic lichen species richness and composition (Phoenix et al., 2006; Suding et al., 2005).Woodland management can affect lichen vegetation due to fragmentation of forests (Boudreault et al., 2008) or alteration of stand structure (such as tree age or distance between trees). Forest management may also cause microclimatic changes in light, humidity, wind or temperature (Campbell and Coxson, 2001; Coxson et al., 2003; Lehmkuhl, 2004; Sillett and Goslin, 1999). Agriculture activities may also influence lichen vegetation through an increase in nutrients supply or pollutants, such as NH_3, that can change bark chemistry and pH (Ruoss, 1999; van Herk, 1999; van Herk, 2001).

Nevertheless effects of management in species and communities are often not straightforward to recognize measure and evaluate. Areas with more intensive forestry activities are usually less species rich due to the disappearance of sensitive species (Boudreault et al., 2000; Dettki and Esseen, 1998; Humphrey et al., 2002; Newmaster and Bell, 2002). Moreover such areas carry usually also a lower lichen abundance or biomass due to less favorable environmental conditions (Dettki and Esseen, 1998; Lehmkuhl, 2004). However this is only true when disturbances are strong enough to cause fatal damage to sensitive species (Rolstad et al., 2001). Low-levels of disturbance can even increment species richness in woodlands (Wohlgemuth et al., 2002). Therefore it is important to understand what levels of disturbance are still favorable for lichen species richness and when they become unfavorable. Most studies done regarding the effect of land-use intensity in lichen communities consider strong land-use intensities (Coxson et al., 2003; Dettki and Esseen, 1998, 2003; Hedenas and Ericson, 2003; Ruoss, 1999; van Herk, 1999; van Herk, 2001) and therefore the effects of low-intensity land-use are not well characterized.

Monitoring the effects of management using lichen biodiversity is an economical way to obtain spatially detailed information which can be crucial for environmental planning and biodiversity policies. When studying the effects of land-use intensity, lichens are frequently divided into eutrophication-tolerant or nitrophytic and eutrophication -sensitive or oligotrophic species (Frati et al., 2007; Pinho et al., 2008b; Rogers et al., 2009; Sparrius, 2007; Wolseley et al., 2006a). A classification of species regarding eutrophication tolerance is available for Italy (Nimis and Martellos, 2008) and was used in this work, because not only all species found here are also found in Italy but also because both countries have a Mediterranean climate.

Here we focus on traditionally managed cork-oak (*Quercus suber* L.) woodlands. *Quercus suber* is restricted to the western Mediterranean region to areas that normally exhibit small temperature amplitudes (Castro et al., 1998). In south-western Portugal *Q. suber* is the dominant tree and cork-oak woodlands have been traditionally managed for centuries. Traditional management includes harvesting of the outer bark (cork) of *Q. suber* every 9 years (Oliveira et al., 2002) and other forestry actions such as the plantation of new *Q. suber* individuals, selective cutting as well as livestock grazing in different intensities. The epiphytic lichen communities of *Q. suber* have been studied by several authors in Portugal and Spain (Carvalho et al., 2002; Fos, 1998; Jones, 1980). However, no study has been done regarding the influence of low-intensity land-use on *Q. suber* lichen vegetation and its implications for biomonitoring dealing with woodland management.

The objective of this work was to determine the effect of traditional low-intensity land-use practices in Mediterranean cork-oak woodlands on epiphytic lichen communities, in order to predict the use of the lichen community as early indicators of the land-use intensity. We hypothesized that lichen functional groups dynamics with a different tolerance to eutrophication, could be considered as early indicators of low intensity land-use changes. Due to the overall rather low management intensities it was not clear if increased management intensity could even lead to an increase in species richness as is predicted by the intermediate disturbance hypothesis (Huston, 1979). Moreover, we consider that lichens are an ideal community to test this hypothesis. Lichens are among the most sensitive communities to disturbances (Cape et al.,

2009) and should therefore respond even to the lowest intensities.

METHODS

Study area

The study was performed in cork-oak woodland near Alcochete (Fig. 1), 25 km east of Lisbon (38.80ºN 8.85ºW; Portugal). In west-Mediterranean region, cork-oaks occur in areas in which the average minimum temperature of the coldest month is above 6º C and the yearly precipitation above 400 mm (Fos, 1998). In Portugal, cork-oak woodlands occupy 7367 km^2 (AFN, 2006). In our study area, all cork oaks are harvested for cork every 9-12 years. Additionally some areas are managed for extensive livestock-pasture. In the study area altitude ranges from 10 to 50 m, the soil is sandy alluvium and eolic deposited. The regional climate can be described as thermomediterranean (Rivas-Martinez and Rivas-Saenz, 2009) which is characterized by dry hot summers and rainy mild winters (climatogram in Fig. 1). The average annual temperature is 17.5 ºC and the average annual precipitation is 600 mm, averages 1931-1960 (IA, 2006).

Sampling design

Four squares of 1 km^2, each one with similar overall land-use intensities, were chosen and within each square 16 sampling plots were sampled (total n = 64 sampling plots). Sampling plots were located at a maximum distance of 8 km of each other and presented the same macroclimate, soil, and altitude. Within each sampling plot we sampled data on the diversity and species composition of epiphytic lichens and we estimated the intensity of land-use.

Lichen richness and abundance

Lichen data were collected between 2001 and 2003, within the EU funded project 'BioAssess'. European level BioAssess results are available elsewhere (Bergamini et al., 2005; Stofer et al., 2006). Lichen diversity was measured using a sampling grid according to a standard protocol (Asta et al., 2002). Within each of the 64 sampling plots 6 *Quercus suber* trees were chosen according to a specific protocol (Scheidegger et al., 2002). We then selected the first six trees for sampling epiphytic lichen communities.

On each tree, lichens were sampled within each of the four main aspects (east, north, south and west) by means of 50 x 10 cm grids divided in five 10 x 10 cm subunits. The grids were placed on the main trunk above the cork-harvest zone. The frequency of each lichen species on a tree was defined as the number of grid cells occupied by that species. Lichen sampling was always carried out in the zone of the trunk that was never harvested for cork. The total area sampled at each plot was 1,2 m^2 (6 trees*4 aspects*500 cm^2 grid).

For each plot, we compiled a species list together with the mean species' frequency, which was based on the frequencies of each species on the individual trees. The following two variables were derived from this species list: i) total species richness per plot; and ii) the total Lichen Diversity Value (LDV) according to (Asta et al., 2002). LDV is the sum of the individual species frequencies per plot and accounts thus for both species richness and the frequency of the species. Additionally, each of these variables was calculated by grouping species according to eutrophication-tolerance.

Regarding eutrophication tolerance an available index which ranges from 1 to 5 (Nimis and Martellos, 2008) was used. Species with the maximum classification of 4 or 5 were considered tolerant (nitrophytic); species with the maximum classification of 1 or 2 were considered sensitive (oligotrophic). The highest eutrophication-tolerance value of each species was used in order to have a measure of the maximum tolerance. Species nomenclature follows Nimis (1993), and for the species not included in this work Clauzade & Roux (1985).

Land-use intensity

Within the cork-oak woodlands, land-use intensity, characterized by pasture and forestry activities, is not homogenously distributed. Grazing intensity was characterized by a pasture index and intensity of forestry by a structure index. Data on intensity of grazing and forestry was available from the landowners, the "Companhia das Lezírias" (agricultural company) and the "Campo de Tiro de Alcochete" (military installations).

We defined the pasture index as follows: Pasture index = Aden*Nrdays*Ai

Aden stands for Animal density (nr unit/ ha), Nrdays stands for the Number of Days with grazing and Ai is an index given to the type of animal present (sheep=1; cow=4).

The forestry index was calculated as follows: forestry index = ∑(Si*Ai)

Si stands for an index attributed to each vegetation type (managed uneven aged woodland=1, managed even-aged forest =2, semi-natural pasture with isolated trees=3, grazed grassland without any trees=4) and Ai stands for the % of the sampling plot area occupied for each type.

Grazing is mainly by cows in an extensive, low-intensity, manner: some of the plots are permanently devoid of animals and in others animal density ranges from 0.07 to 0.24 animal ha^{-1}.The number of grazing days ranges from 45 to 60 days per year. The forestry index reflects the impact of forestry activities in the vegetation, namely selective tree cutting, shrubs removal and cork harvest. Higher values of the structure index indicate dominance of open woodlands, lower values dominance of closed woodlands.

A previous analysis studied the separate effect of pasture and forestry indexes on lichen variables (not shown). But since the pasture index and the structure index were significantly correlated ($R = 0.58$, $p < 0.001$) it was not possible to interpret them separately most probably because livestock influenced vegetation structure. Consequently, the two indexes were standardized and summed, originating a Land Use Intensity index (LUI) which thus assumes additivity of the two indexes to describe land-use intensity.

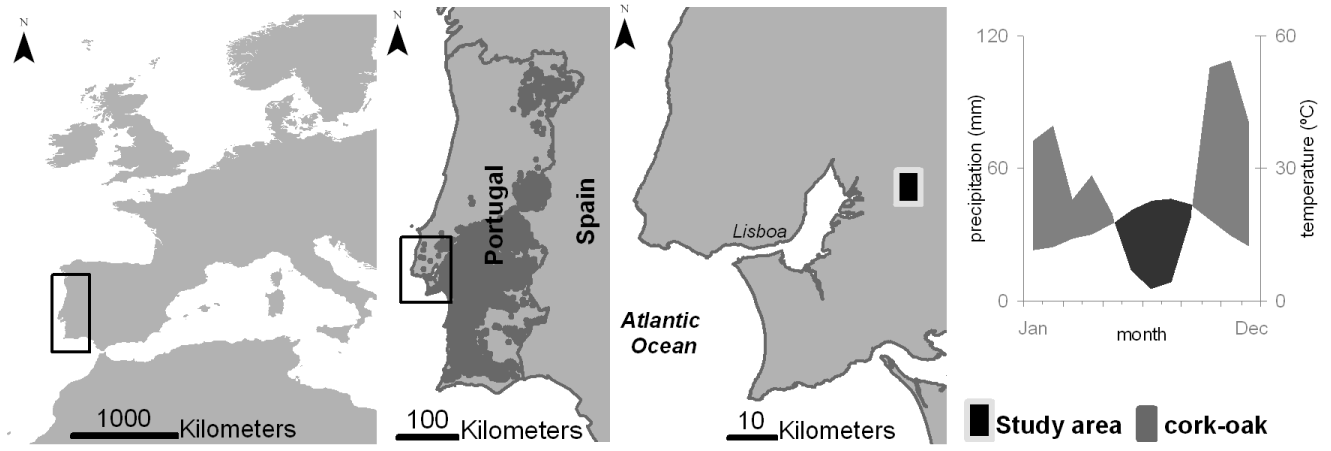

Figure 1: General location of the study area in Europe and Portugal showing the current distribution of cork-oak (Quercus suber L.) in Portugal (AFN, 2006) and a climatogram for the study area (weather station of "Monte da Caparica", average values from 1985 to 2007) highlighting the xeric period (darker area).

Data analysis

Species frequency data were analyzed by Principal Component Analysis (PCA). A linear method was chosen after running a DCA on the species data (not shown) showing that the gradient lengths were smaller than 1.2 (for the first two axes), thus suggesting a linear relationships between species and underlying environmental variables (ter Braak and Smilauer, 2002). For the species analysis we considered only the species present in 5 or more sampling plots, thus excluding 37 out of a total of 86 species and used the remaining 49 species. We did this in order to ensure that the distribution of

species in the analysis was due to a true (that is, re-verifiable) species response to the environment and not to a random or very specific association of uncommon species to a few sampling sites. The Land Use Intensity index was plotted in the species PCA space (as passive variable) in order to observe its relationship to the species distribution.

Linear regressions were used to analyze the relationship between LUI and i.) the site scores of the first factor of the PCA; and ii.) species richness and LDV. Values of species richness were square root transformed and LDV values were log transformed before analysis to improve the distribution of the regression residuals. All regressions were also performed using relative values of the dependent variables but the results were not different and are therefore not shown. All analyses were done in Statistica (StatSoft, 2004).

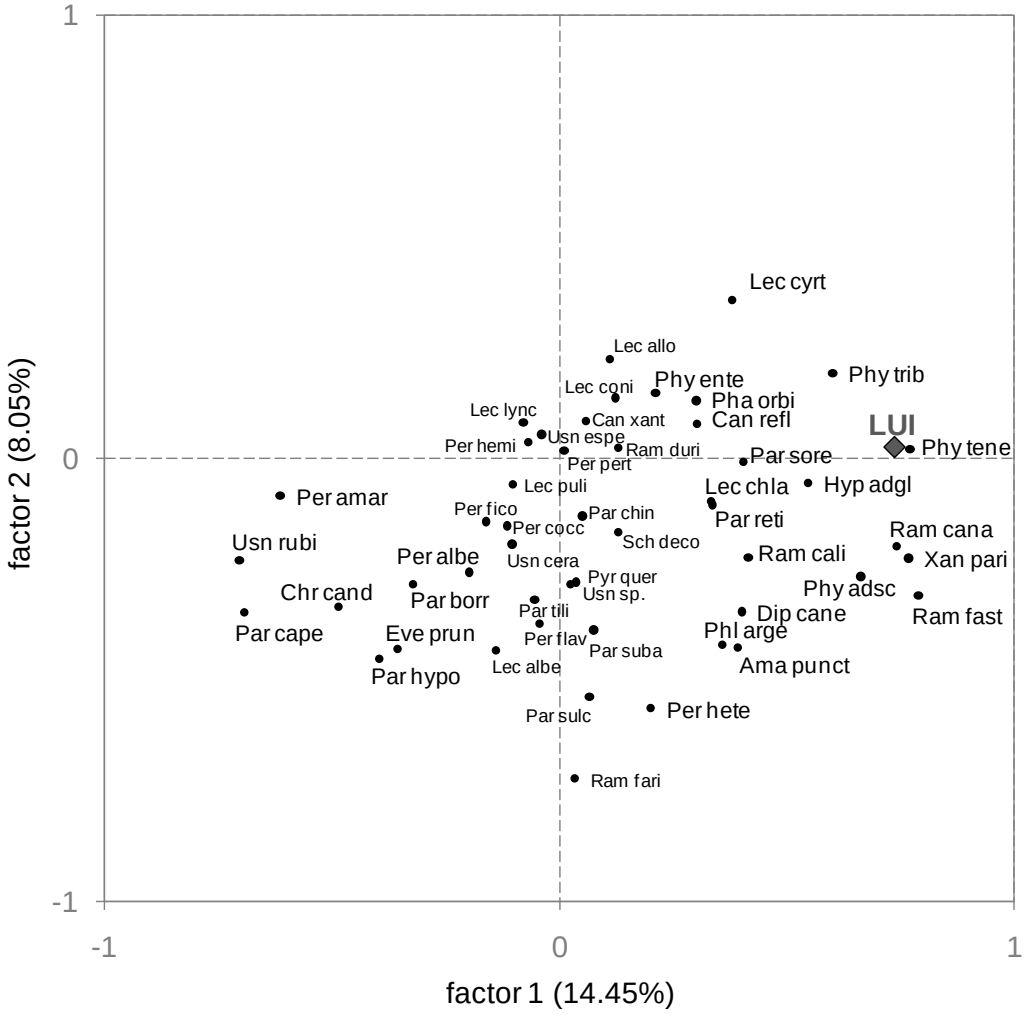

Figure 2: Plot of two first factors of a PCA of species abundance, only for species occurring in five or more plots. The Land-Use intensity index was plotted in the graph as passive variable. Species names, left to right: *Usnea rubicunda, Parmelia caperata, Pertusaria amara, Chrysothrix candelaris, Parmelia hypoleucina, Evernia prunastri, Parmelia borreri, Pertusaria albescens, Pertusaria ficorum, Lecanora albescens, Pertusaria coccodes, Usnea ceratina, Lecanora pulicaris, Lecanographa lyncea, Pertusaria hemisphaerica, Parmelina tiliacea, Pertusaria flavida, Usnea esperantiana, Pertusaria pertusa, Usnea sp., Ramalina farinacea, Pyrrhospora quernea, Parmotrema chinense, Candelariella xanthostigma, Parmelia sulcata, Parmelia subaurifera, Lecanora allophana, Lecanora conizaeoides, Ramalina duriaei, Schismatomma decolorans, Pertusaria heterochroa, Physconia enteroxantha, Phaeophyscia orbicularis, Candelariella reflexa, Lecanora chlarotera, Parmelia reticulata, Phlyctis argena, Lecania cyrtella, Amandinea punctata, Diploicia canescens, Parmelia soredians, Ramalina calicaris, Hyperphyscia adglutinata, Physcia tribacioides, Ramalina fastigiata, Ramalina canariensis, Xanthoria parietina, Physcia tenella, Physcia adscendens.*

RESULTS

Species and community response to land-use intensity

The two first factors of the PCA (Fig. 2) represented 22.5% of the total species variance (first axis: 14.5%, second axis: 8%). Species regarded as eutrophication tolerant (Nimis and Martellos, 2008) were associated to the positive portion of the first axis, species regarded as eutrophication sensitive (Nimis and Martellos, 2008) to the negative portion of that axis (Fig. 2). In fact, nitrophytic species such as *Physcia tenella*, *Ramalina canariensis*, *Xanthoria parietina* and *Ramalina fastigiata* were strongly and positively related to the first axis. On the other hand, oligotrophic species such as *Usnea rubicunda*, *Parmelia caperata* and *Pertusaria amara* were negatively related to this axis. The LUI index, that reflects the intensity of forestry and pasture activities, was clearly related to the first factor (Fig. 2). In fact, there was a significant linear correlation between the PCA site scores of the first factor, that reflect the main species distribution within the studied gradient, and the LUI of each site (R^2 =0.55, $p < 0.001$, Fig. 3).

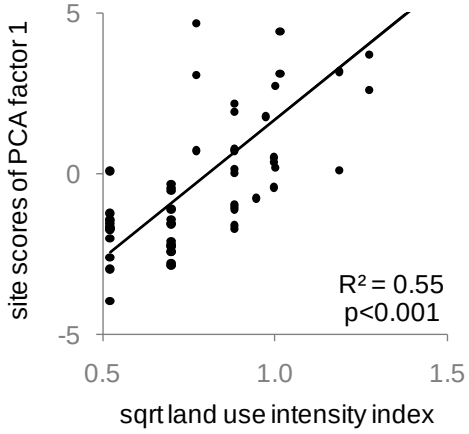

Fig. 3. Linear regression of the PCA site scores of the first factor of a PCA (Fig. 2) against the land-use intensity index, which reflects the intensity of forestry and pasture activities.

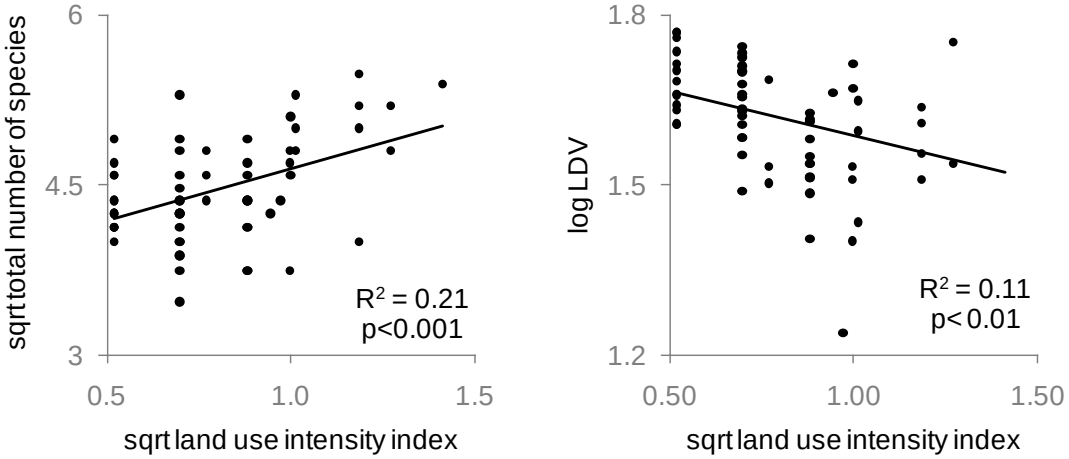

Figure 4: Linear regressions of total species richness and total LDV on the Land Use Intensity index.

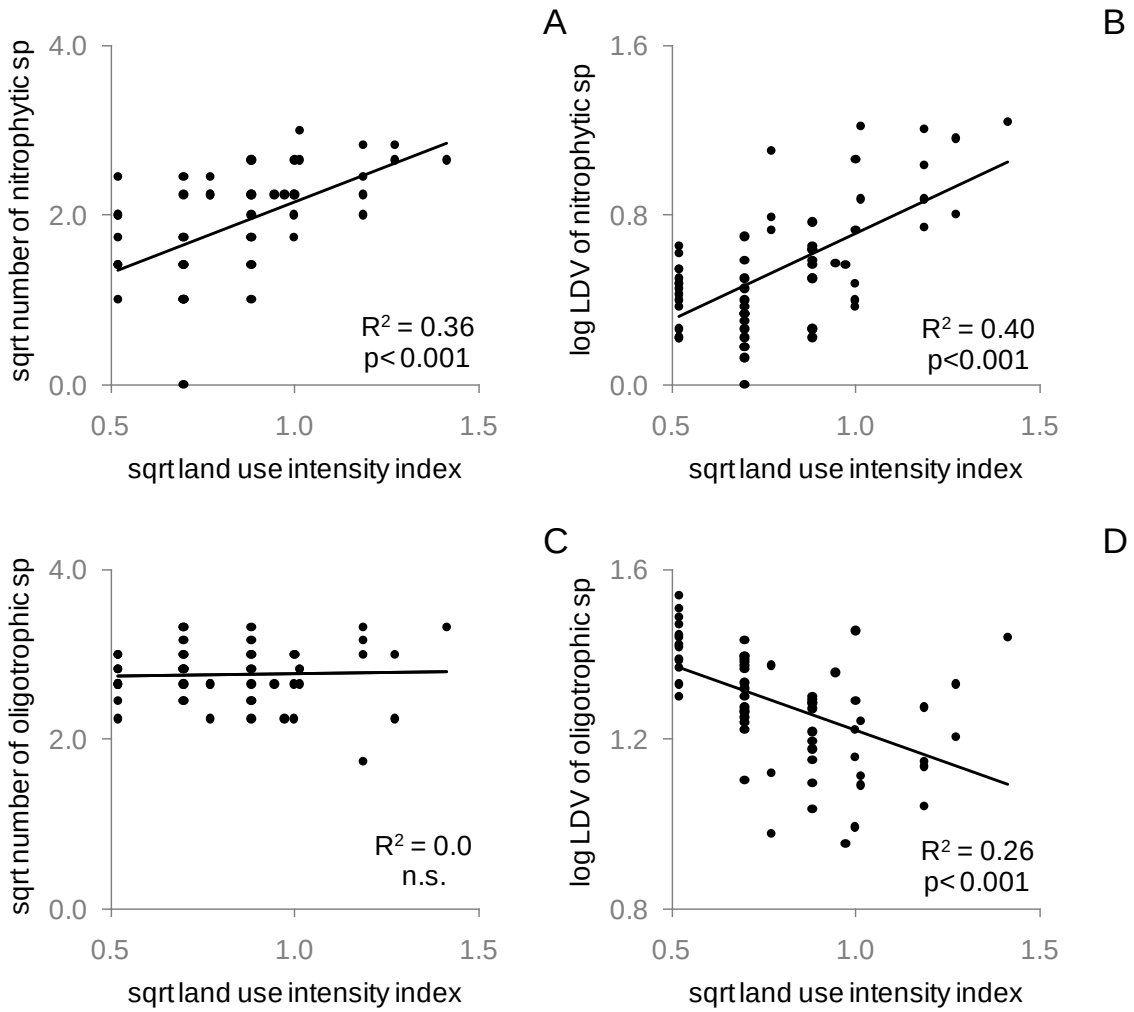

Figure 5: Relationship between the land use intensity index and the richness and LDV of nitrophytic and oligotrophic species by means of linear regressions.

Regressions of the two lichen-variables that account for the entire lichen community, total species richness and of total LDV, on the LUI index were highly significant (Fig. 4). However, while total species richness was positively related to LUI, total LDV decreased with increasing LUI.

Considering nitrophytic species only, both the richness and the LDV of these species increased significantly ($p < 0.001$, Fig. 5A and B). The richness of oligotrophic species showed no relationship with LUI (Fig. 5C), but the LDV of these species was negatively related to LUI ($p < 0.001$, Fig. 5D).

DISCUSSION

Here we have shown that lichen species richness and composition responded to an intensity gradient of management in a Mediterranean agro-ecosystem (Fig. 3) in accordance to what was found in other works (Aragon et al., 2010; Pinho et al., 2009; Wolseley et al., 2006b), showing its potential as ecological indicators. However it is the first time that an increase in total species richness is reported under increasing land-use intensity (Fig. 4), probably because our plots were clearly situated within a low-intensity land-use gradient. Our results fit the intermediate disturbance hypothesis (Huston, 1979; Roxburgh et al., 2004) which predicts maximum diversity under intermediate disturbance frequency. Previous authors have found that lichen species richness could be maintained (Nascimbene et al., 2007) or decreased under a land-use gradient. An increase in land-use intensity is usually associated to the loss of species, especially the rare and sensitive ones (Belinchon et al., 2007; Boudreault et al., 2000; Dettki and Esseen, 1998; Humphrey et al., 2002; Lehmkuhl, 2004; Newmaster and Bell, 2002). We suggest that the highest intensity that was studied must be lower than the threshold level of LUI above

which total species richness tend to decrease. At greatest LUI, namely increasing the cattle density, a decrease in oligotrophic species richness and abundance is expected until their complete disappear (Pinho et al., 2009).

A closer look to the functional diversity of the epiphytic lichens showed that functional groups with different tolerance to eutrophication responded in dissimilar ways, giving a better insight on the lichen community response (Fig. 5). The richness and abundance of eutrophication-tolerant species increased with increasing LUI (Fig. 5A, B). However, abundance of sensitive species decreased with increasing land-use intensity but without changes in richness (Fig. 5C, D). Therefore, the observed total species richness increase could be attributed to an increase of nitrophytic species while at the same time oligotrophic species richness was maintained (Fig 5). A recent meta-analysis of 120 individual comparisons of species richness between unmanaged and managed forests throughout Europe (Paillet et al., 2010) concluded that lichen richness was negatively affected by forest management. Cyanolichens are commonly reported as the species that disappear under increasing land-use intensity (Aragon et al., 2010; Belinchon et al., 2007). Therefore in our case the increase in total species richness could be related not only to the low-intensity of the management activities but also to the fact that cyanolichens were absent for our site. However, cyanolichens are very rarely reported from cork-oak woodlands (Fos, 1998) and therefore the observed species in our study are likely close to the maximum potential lichen vegetation of this ecosystem.

The results allowed us to exploit the potential factors associated to land-use intensity that could drive the changes in lichen communities. Grazing areas are known to experience higher levels of, NH_3 whose effect on lichens communities has already been shown (Frati et al., 2008; Pinho et al., 2009). Although the density of cattle in our study area is low, an increased concentration of ammonia is expected even with small number o animals and could therefore be one of the probable causes for the changes in functional groups observed (Fig. 5). Atmospheric ammonia at low concentrations might have a fertilizer effect increasing the growth of lichens which are tolerant to N (Gaio-Oliveira et al., 2001). Another possible cause could be the forestry activities that induce lower humidity and higher light availability (Jactel et al., 2009), thus favoring the occurrence of the most tolerant species. Additionally, dust could also be the cause of the changes observed because it was shown to have a negative impact in lichen physiology (Branquinho et al., 2008) and was related to a promotion of nitrophytic species (Pinho et al., 2008b).

An important application of the obtained results is the possibility to use lichens as integrated indicators of the impact of low-intensity land-use. Most lichen-variables tested presented significant relations to LUI (Fig 4, 5). Global lichen-variables, such as total species richness and total LDV have proved to be suitable to monitor the changes associated to industrial or urban pollution (Pinho et al., 2008a; Purvis et al., 2003). However, when monitoring the impact of agriculture lichen variables based on eutrophication tolerance are preferred (van Herk, 1999). For instance, one can report no changes in environmental conditions due to the high species number found, although most of the species present can be nitrophytic (Van Dobben and Ter Braak, 1998). As in previous studies dealing with the effect of land-use intensity (Pinho et al., 2008a; Pinho et al., 2009) the variables based in species functional groups were necessary to better understand the effects of land-use in lichen communities. An important outcome is that lichens communities were shown to be sensitive to very low-intensity land-use. Therefore lichens can be used to monitor the impact of even the lowest-intensity management activities. Lichen variables based in abundance such as LDV could be preferred over species richness, because the effects of land-use intensity could be observed in both sensitive and tolerant species LDVs before an effect was seen on sensitive species richness (Fig. 5).

CONCLUSIONS

We showed that traditional low-intensity land-use can sustain and even promote high levels of lichen species richness. Traditional, low-intensity land-use shifted the lichen communities towards more nitrophytic species. Additionally, although oligotrophic species become less abundant, the increased land-use intensity did not decrease sensitive species richness. Therefore, the results highlight the ability of traditional low-intensity land-use for maintaining high species diversity.

Lichen communities could be used for monitoring low-intensity land-uses in agro-ecosystems, given the fact that lichen community dynamics responded to rather small changes in management intensity. Additionally the results have shown that the abundance of species grouped accordingly to eutrophication-tolerance were sensitive to low-intensity land-use. This result confirmed the importance of functional-traits based indicators for monitoring changes in ecosystems and also the possibility of using lichens as early-indicators of the impacts of low intensity land-use.

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chapter 5

05 | Using lichen functional-diversity to assess the impact of atmospheric ammonia in Mediterranean woodlands

ABSTRACT

Atmospheric ammonia (NH_3) is currently recognized as one of the main drivers for ecosystem changes worldwide - including biodiversity loss. There is a need to have objective and universal tools to determine and compare the early effects of atmospheric NH_3 across ecosystems. Aiming at providing a robust and accurate method to map the effects of atmospheric NH_3 in ecosystems, we determined its influence in lichen species and functional-diversity. To do so we evaluated the spatial and temporal patterns of atmospheric NH_3 concentrations, emitted from a point-source, during one year period in a cork-oak Mediterranean woodland. The distribution of lichen species was mostly explained by the atmospheric NH_3 concentrations measured. The tolerance to atmospheric NH_3 of individual lichen species based on expert knowledge available from literature could be quantitatively and explicitly tested, for the first time, against measures of atmospheric NH_3 under field conditions concentration. The results have shown that most species are well classified for NH_3 tolerance with exception of *Lecanora albella* and *Chrysothrix candelaris* that were previously considered oligotrophic and were reclassified as mesotrophic. As a response to increasing NH_3 concentrations there was a complete replacement of oligotrophic by nitrophytic communities. The geostatistical analysis of these variables showed a spatial model with low non-spatial variance, indicating high robustness of these variables as ecological-indicators of the effect of NH_3 concentration. This result greatly supports the use of functional-diversity variables based in functional traits related to atmospheric NH_3 tolerance, such as LDV of oligotrophic and nitrophytic species.

INTRODUCTION

Nitrogen (N) pollution is considered not only a major threat to global biodiversity but also one that is expected to increase worldwide (SCBD, 2006). The effect of N in worldwide ecosystem functions at a global scale is highlighted by recent works (Rockstrom et al., 2009). These authors considered that the rate of interference with the N cycle has already passed a safety boundary, beyond which negative impacts on human activities could occur at continental or global scales. Most N emitted to the atmosphere is emitted as atmospheric ammonia (NH_3) (Robarge et al., 2002). The main anthropogenic sources of atmospheric NH_3 are agricultural activities, mainly intensive farming with fertilizers and intensive animal husbandry (EPER, 2004; Galloway et al., 2003). Livestock housing facilities are major point-sources of NH_3. The concentrations of NH_3 near those facilities are high, decreasing exponentially with distance, reaching background values after a 1-2 km (Pitcairn et al., 2003; Pitcairn et al., 1998; Sutton et al., 1998). However little is known about the spatial and temporal patterns of dispersion of NH_3 under a Mediterranean climate. Moreover, the effects of atmospheric NH_3 in ecosystems is currently an important topic of research and its impacts are increasingly being detected in several ecosystems worldwide (Aber et al., 2003; Bobbink et al., 2010; Cape et al., 2009a; Erisman et al., 2003; Krupa, 2003; Purvis et al., 2003), being one of the most important biodiversity loss (Phoenix et al., 2006; Suding et al., 2005). Therefore there is a strong need to have reliable tools to assess the effect of atmospheric NH_3 levels in ecosystems.

Ecological indicators are any measurable characteristic of the structure, composition or function of ecosystems (Niemi and McDonald, 2004) and have been widely applied in many problems where it is important to assess the effect of natural and anthropogenic disturbances. Ecological indicators integrate the effects of disturbances and provide information with high spatial resolution. Therefore, their use is especially important in areas

where there is a lack of chemical measures, as in most Mediterranean areas on what regards the measures and the effects of atmospheric NH_3 (Cape et al., 2009b).

Lichens are symbiotic organisms widely used as ecological indicators to monitor the effects of environmental changes (Augusto et al., 2009; Cislaghi and Nimis, 1997; Geiser and Neitlich, 2007; Giordani et al., 2002). Additionally, lichens have been reported to be among the most sensitive organisms to atmospheric NH_3 at the ecosystem level (Cape et al., 2009a; van Herk, 1999; Wolseley et al., 2006). Epiphytic bryophytes, although a very sensitive group (Larsen et al., 2007; Mitchell et al., 2005), are not so widespread, particularly in Mediterranean dryer areas. Some previous works have related the lichens with the atmospheric NH_3 (Sparrius, 2007; Van Dobben and Ter Braak, 1998; van Dobben and ter Braak, 1999; van Herk, 1999; Wolseley et al., 2006). However, most of these works were performed in non-natural ecosystems such as farms and in non-Mediterranean areas. In Mediterranean areas the works done on this subject (Frati et al., 2008; Frati et al., 2006; Frati et al., 2007) used short-time measures of atmospheric NH_3 (less than one month period) and only a few number of samples that could not be modelled spatially. Under increasing eutrophication it has been shown that the richness and abundance of nitrophytic species increases and that of oligotrophic decrease. This occurs because species have different sensitivities to eutrophication, which allows grouping species according to eutrophication-tolerance (Suding et al., 2005). This type of expert-knowledge classification is available for Mediterranean lichens species (Nimis and Martellos, 2008), but has never been tested against a gradient of measured NH_3.

For spatial modelling of lichen diversity as an ecological indicator, most studies consider the value of a site that results from the average value of c. 12 nearby trees (Asta et al., 2002; Pinho et al., 2008b). This procedure is advisable because for most studies the main environmental factor under study presents a high spatial continuity, thus being homogenous over space and consequently nearby trees, e.g. located at less than 100m, will respond in the same way. For example variograms based on lichen diversity have shown a spatial range of 80 km in a region influenced by multiple factors, including industrial pollution (Pinho et al., 2008a). However NH_3 concentration is known to change in very short distances, most variations occurring within less than 1km from source (Loubet et al., 2009; Sutton et al., 1998). Thus averaging a number of trees grouped a priori may result in unacceptable uncertain at short range, leading to variograms with great nugget effect and thus with poor prediction capability. Therefore a new approach must be implemented when dealing with lichen diversity information in space having in mind the great variation in space of the deposition of atmospheric NH_3.

Our objectives were to study, in Mediterranean-type ecosystems, the spatial and temporal characteristics of the dispersion of atmospheric NH_3 and also to determine its effects on lichens species and communities, in order to validate the expert-knowledge classification of lichens into functional groups. By doing so we aimed at providing a robust and accurate method to map the effect of atmospheric NH_3 in Mediterranean-type ecosystems. For that we have selected one cow-barn surrounded by Mediterranean semi-natural cork-oak woodland a Natura 2000 habitat (ALFA, 2004).

MATERIALS AND METHODS

Study area

The study area is Mediterranean woodland, located in Portugal, 30 km East of Lisbon (Fig. 1). In an edge of the woodland, approximately 200 cows are permanently housed in a single barn with 800 m^2. To the South of the barn we can find cork-oak woodland (*Quercus suber* L.). This woodland presents an homogeneous land-use intensity and structure, thus avoiding the possible confounding effect of dust and light reported by other authors in Mediterranean areas (Loppi and Pirintsos, 2000). Some areas of extensive pasture exist to the South-West of the woodland, mainly for sheep. Eleven Km to the West there are two main motorways and 2.5

Km to the north a military aviation training facility. Soils of this region are sandy alluvium and eolic deposited, the average annual temperature is 17.5 ºC and the average annual precipitation is 600 mm (averages 1931-1960) (IA, 2006). The study area presents a typical Mediterranean climate (Thompson, 2005) and is located in the thermomediterranean climatic belt (Rivas-Martinez and Rivas-Saenz, 2009), characterized by dry hot summers and rainy mild winters. Sample was carried out in the woodland located to the south of the barn (Fig. 1) on which NH_3 and lichens were sampled (Fig. 1).

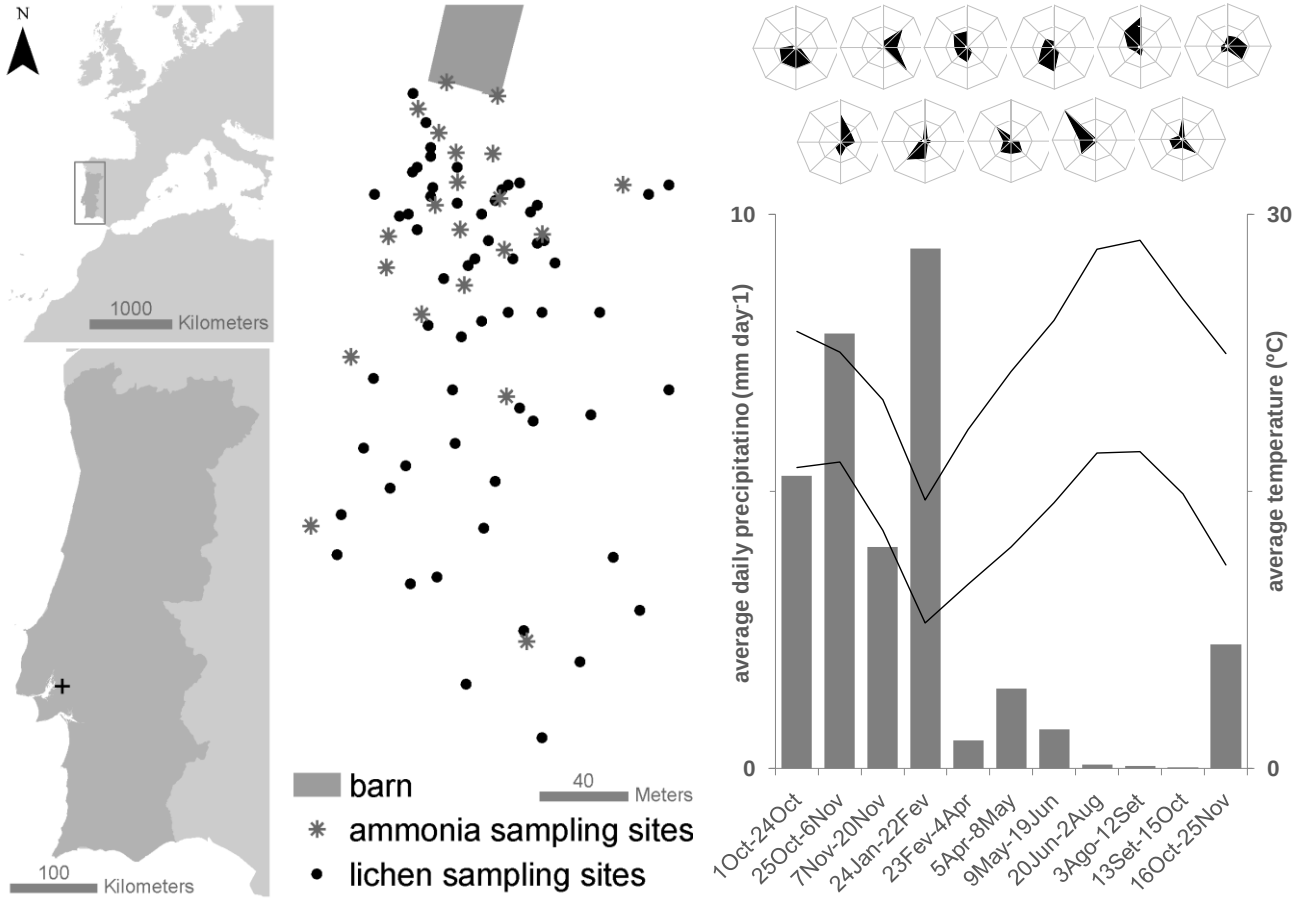

Figure 1: **left**: location of the study area in Europe and in Portugal (+); **centre**: location of the atmospheric NH_3 sampling sites (n=21) and lichen sampling sites (n=74, some are not visible); **right**: climate during the study time; climate information is from the nearest meteorological station (23 km to the West) and is presented for each sampling period: average daily precipitation (rectangles), averages of maximum and minimum temperatures (lines), wind direction in % hours with wind coming from each direction (the full octagon is 50%).

Ammonia and lichen sampling

The atmospheric NH_3 concentrations were measured on eleven sampling periods from October 2006 to November 2007 (2-weeks measurements from October to December 2006, 4 week continuous measurements for the remaining time). High sensitivity ALPHA (Adapted Low-cost Passive High Absorption) passive diffusion samplers were used (Tang et al., 2001). Measurements were carried at 21 sampling locations (2 ALPHA samplers at each point) downwind of the cattle barn (Figure 1). Mean distance between NH_3 samplers was 28.1 m. Most sampling points were located within 200 m of the barn, since that is where the largest variation in concentrations was expected (Sutton et al., 1998). Ten field blanks were randomly distributed amongst the sampling points. Ammonium ($N\text{-}NH_4^+$) was analyzed using a modified Berthelot reaction (Cruz and Martins-Loução, 2000) using a spectrophotometer Tecan Spectra Rainbow A-5082. At four sampling locations, additional ALPHA

samplers provided by CEH were set up in parallel to those prepared by University of Lisbon for quality assurance purposes. Results were compared with those obtained for the parallel CEH samples that were analyzed independently by CEH using the AMFIA (Ammonium Flow Injection Analysis) system (Sutton et al., 2001). The NH_3 concentrations determined with the ALPHA samplers were calibrated against measurements obtained with a CEH DELTA (Denuder for Long-Term Atmospheric sampling) system. A good correlation was observed between the different methods used to determine NH_3 concentration.

Epiphytic lichens were sampled on the main trunk of cork-oak trees using a sampling grid accordingly to a standard protocol (Asta et al., 2002; Scheidegger et al., 2002). Seventy-four trees were selected for the survey (Fig. 1), taking into account several criteria (Pinho et al., 2004). Mean distance between trees was 14.8 m. In each tree, a 50 x 10 cm grid, divided into five 10 x 10 cm subunits was placed on the four main aspects the tree trunk above the cork-harvest zone. Species frequency was noted as the number of squares it occurred. A Lichen Diversity Value (LDV) (Asta et al., 2002) was calculated for each tree, as the sum of the frequencies of all species. This index therefore accounts both for species number and frequency. Besides individual species frequency and total LDV (using all species), we calculated three functional-diversity variables: i.) LDVoligo (considering nitrogen-sensitive species); ii) LDVnitro (considering nitrogen-tolerant species); and LDVmeso (considering the intermediate species). These functional-diversity variables were calculated grouping species taking into consideration an eutrophication-tolerance index (Nimis and Martellos, 2008). The highest classification for each lichen species was considered: species classified as 4 and 5 were considered nitrophytic (LDVnitro), 1 and 2 as oligotrophic (LDVoligo) and 3 as intermediate (LDVmeso). These threshold values of each class (or functional group) were chosen by us, in order to unsure a good distribution of LDV values on each class.

Geostatistical analysis and interpolation of lichen functional-diversity variables

The site of sampling lichens and ALPHAs was not the same (Fig. 1). In order to relate lichen-variables to the atmospheric NH_3 measures at each sample location we interpolated all lichen-variables for the entire study area, using each tree as a sample. Using this interpolated map the values of the lichen-variables were determined at the NH_3 sampling locations. A spherical model including anisotropy and nugget effect was fitted to the data. This model was used for two purposes. Firstly it was used to interpolate lichen-variables using ordinary Kriging, considering the 6 nearest trees. Additionally it was used to test the robustness of the lichen functional-diversity variables. This was evaluated by considering the nugget effect, which was interpreted as the proportion of variance without a spatial structure, normally due to the effects of sampling site factors on the data (e.g. tree characteristics).

Data analysis

In order to analyse lichen community composition within the studied area we performed a Principal Components Analysis (PCA) of the species frequency data at the tree level (N=74). To check the accuracy of the classification used (Nimis and Martellos, 2008) we correlated the maximum value given to each species on the eutrophication-tolerance index (ranging from 1 to 5) to the species PCA scores using Spearman Rank Order Correlations. To test if NH_3 concentration could be the main driving force for species ordination on the PCA, we related site scores to the its concentration (N=18). The relation between lichen functional-diversity variables (LDVs) and atmospheric NH_3 concentration was also assessed by linear correlations (N=18). All correlations were performed using the values of lichen-variables (PCA-scores, LDVtotal, LDVnitro, LDVmeso and LDVoligo) estimated in the NH_3 sampling locations as explained above. These estimated lichen-variables were plotted against the observed values of the average NH_3 concentration for each ALPHA location

(n=18, samplers outside the spatial range of the trees sampling locations were not used, Fig. 1). A curve was fitted to each biplot minimizing the squared residuals, y=a*ln(x)+b (for positive relations) and y=a*x-b (for negative relations).

The robustness of the lichen functional-diversity variables as indicators of the impact of NH_3 was evaluated by the proportion of the nugget effect of each lichen-variable. This parameter was determined from the geostatistical analysis detailed above. The accuracy was evaluated by the strength of the relation between lichen-variables and the atmospheric NH_3 concentration.

Correlation plots were presented without transformations, showing an exponential decrease or increase, of lichens-indicators with higher NH_3 concentrations. These plots were presented with a R^2 value, the square residuals, as a measure of the relation's strength. More importantly, the regression assumptions were verified in biplots using the transformed data. Normality of the residuals approached a normal distribution, although care must be taken, because a low number of samples were available. The homogeneity of variance of the residuals, which was greatly improved by the transformation, was found to meet the assumptions. All Geostatistics and GIS operations and output maps were done with ArcMap 9.3 (ESRI, 2008). Correlations and function fitting were performed with Statistica (Statsoft, 2008).

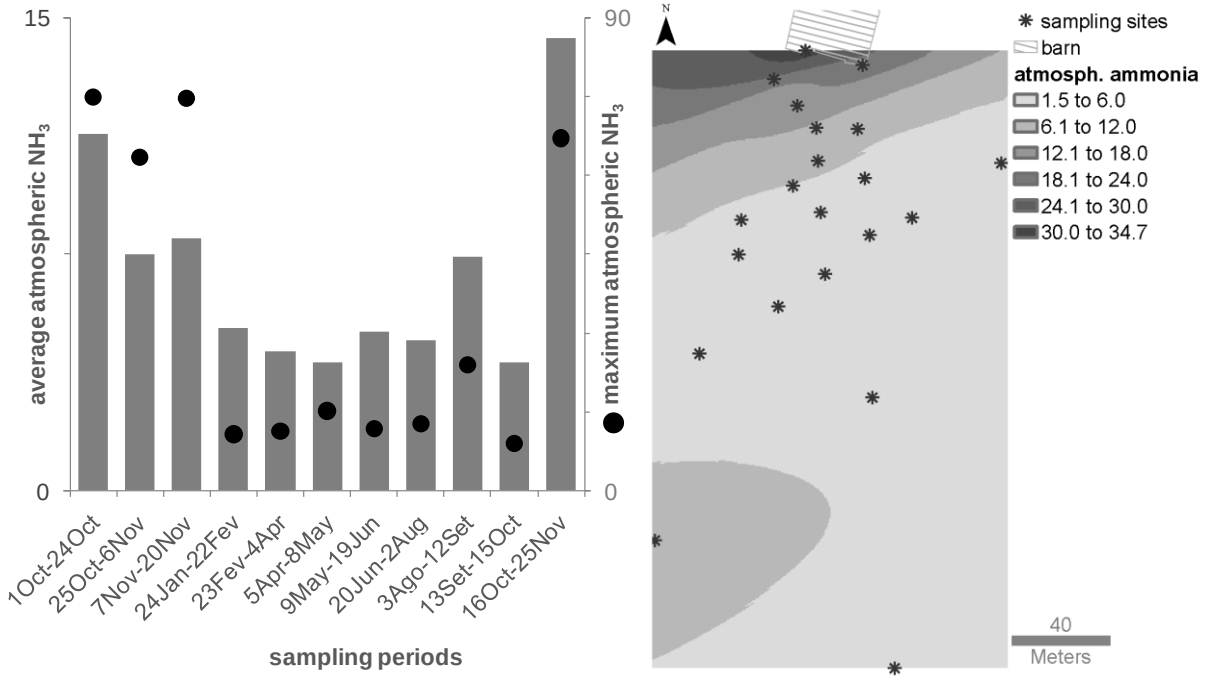

Figure 2: **left**: plot of the ammonia concentration on each sampling period, showing the average and maximum values (averages off all sampling sites, N=21); **right**: mapping of the interpolation of ammonia concentrations for the study area (average of the 11 time periods).

RESULTS

Atmospheric ammonia concentrations

The average NH_3 concentration values ranged between 34.7 μg m^{-3} and 1.51 μg m^{-3} (Fig 2, right), with the highest values observed near the source and decreasing exponentially with distance from the cow-barn. The concentration of NH_3 varied greatly between sampling periods: the concentration of NH_3 measured at the sampling site with the highest mean concentration (34.7 μg m^{-3}) ranged between 8.04 μg m^{-3} and 74.98 μg m^{-3} whereas the one measured at the site with the lowest concentration (1.51 μg m^{-3}) ranged between 0.37 μg m^{-3} and 2.78 μg m^{-3}. Additionally, by averaging all sampling sites for each sampling period (Fig. 1, left), we could observe large variations between sampling periods,

with the highest concentrations observed in autumn (October to November).

Effect of atmospheric NH_3 in lichen species

A total of 28 epiphytic species were found in the studied area. Of those 8 presented a crustose growth form, 6 foliose-broad-lobed, 5 foliose narrow-lobed and 9 fruticose (Nimis and Martellos, 2008). The species ordination in a PCA (Fig. 3) has shown that the first factor extracted accounted for 21.5% of species variance and grouped in the positive side species previously classified as oligotrophic and on the negative side species classified previously as nitrophytic by (Nimis and Martellos, 2008). There were two exceptions regarding the adequacy of this classification: *Lecanora albella* and *Chrysothrix candelaris*. The expert classification sorted these species in the eutrophication index with the value of 1, indicating that these species should be restricted to sites with no eutrophication (Nimis and Martellos, 2008). However both species were located in a mid position on the PCA ordination (Fig. 3), which was not compatible with the previous classification. Thus they were reclassified as being mildly tolerant to eutrophication (with a maximum value of 3 in the eutrophication-tolerance index (Nimis and Martellos, 2008), thus in this work regarded as mesotrophic) and all subsequent calculation of LDV has taken this into consideration.

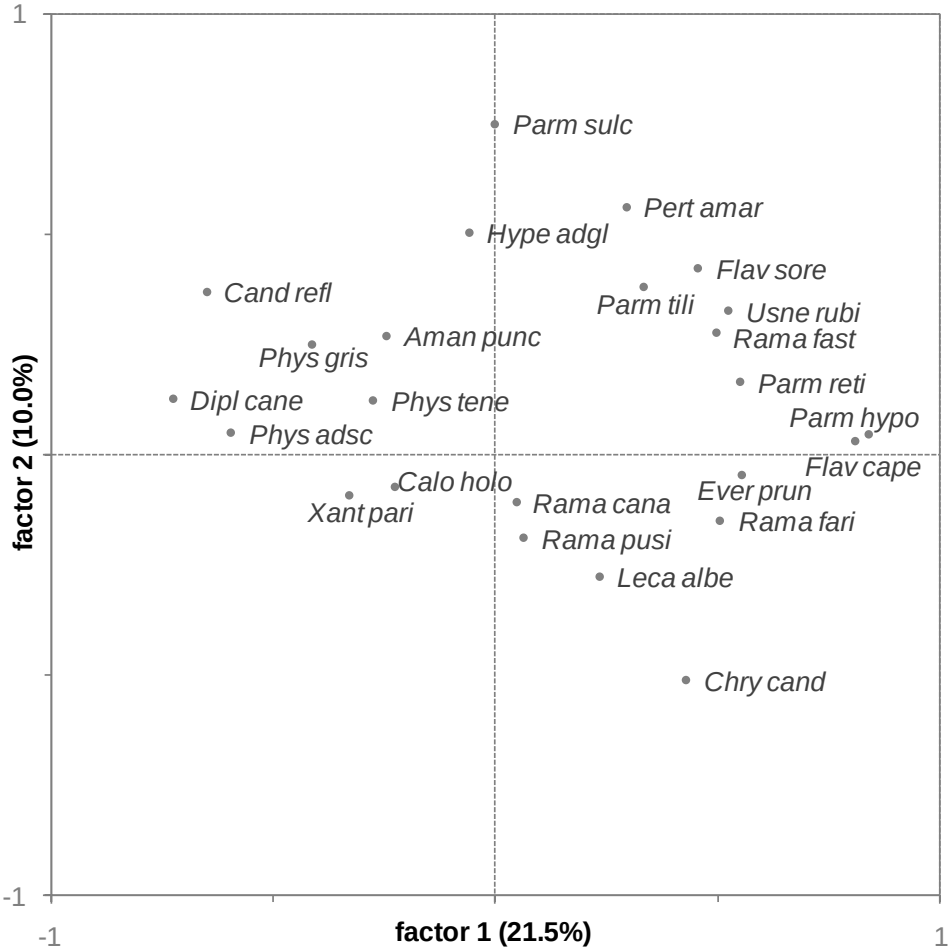

Figure 3: Representation of the first two factors of a Principal Components analysis of species frequencies. Species names, left to right: *Diploicia canescens*, *Candelariella reflexa*, *Physcia adscendens*, *Physconia grisea*, *Xanthoria parietina*, *Physcia tenella*, *Amandinea punctata*, *Caloplaca holocarpa*, *Hyperphyscia adglutinata*, *Parmelia sulcata*, *Ramalina canariensis*, *Ramalina pusilla*, *Lecanora albella*, *Pertusaria amara*, *Parmelina tiliacea*, *Chrysothrix candelaris*, *Flavoparmelia soredians*, *Ramalina farinacea*, *Ramalina fastigiata*, *Evernia prunastri*, *Usnea rubicunda*, *Parmotrema reticulatum*, *Flavoparmelia caperata*, *Parmotrema hypoleucinum*. N=74.

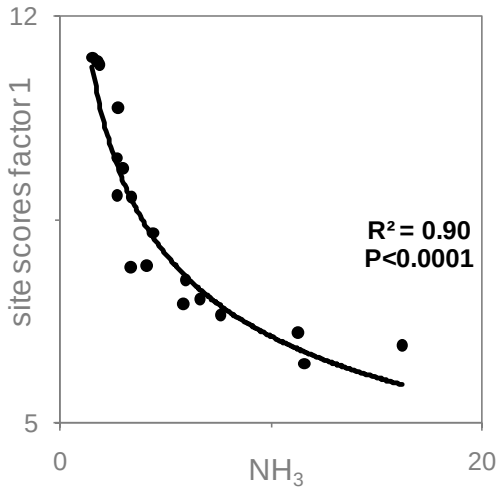

Figure 4: Correlation between average NH_3 concentration and site scores of factor 1 in the PCA analysis (Fig. 3). N=18. Atmospheric NH_3 concentrations in $\mu g\ m^{-3}$.

Spatial analysis of lichen functional-diversity variables

Firstly we performed a variogram analysis of each lichen variable, which allowed modelling their spatial structure (Table 1). Because atmospheric NH_3 concentration is known to change rapidly over space (Sutton et al., 1998), what was confirmed here (Fig. 2), we used lichen-level data to analyse and then interpolate the lichen-diversity variables, rather than averaging a certain number of nearby trees chosen a priory. As a consequence, in the interpolation procedure, the weight of trees located near the point to be estimated was always higher than those located far.

The spatial analysis of lichen-variables (Table 1) showed that all lichen-variables had similar anisotropy (Table 1), with the main orientation to the North / North-East direction and with the main range c. 2 times greater than the minor range. The major difference between the lichen-variables was the relative importance of the nugget effect (Table 1): i) LDVtotal showed a nugget effect accounting by more than half of the total variance; ii) LDVnitro an intermediate weight of the nugget effect (c. 1/3 of total variance); iii) LDVoligo and LDVmeso the smallest nugget effect.

Total LDV varied between 60 and 11, with the lowest values observed at 50 m to the South of the barn (Fig. 5). The lichen functional-diversity variable LDVmeso ranged from 0 to 37 and presented a pattern similar to LDVtotal, but the highest values were observed c. 150 to the south-east of the barn (Fig. 5). The variable LDVoligo ranged from 0 to 29, with the lowest values observed near the barn and the highest ones far from it while LDVnitro presented an opposite spatial distribution and ranged between 0 and 32 (Fig. 5).

Table 1: Characteristics of the model adapted to each lichen variable after variogram analysis. Major/minor range is indicator of anisotropy in the variables (1 indicating no anisotropy); main direction is the direction of higher spatial continuity (0º indicating North); nugget/total variance is the proportion of non-spatially structured variance in the data (0 indicating all variance with a spatial structure, 1 the opposite). N=74 for each lichen-variable.

variable	major / minor range	main direction	nugget / total variance
LDVtotal	2.63	19.2°	0.54
LDVnitro	2.25	52.2°	0.33
LDVmeso	2.55	19.8°	0.15
LDVoligo	1.99	54.1°	0.14

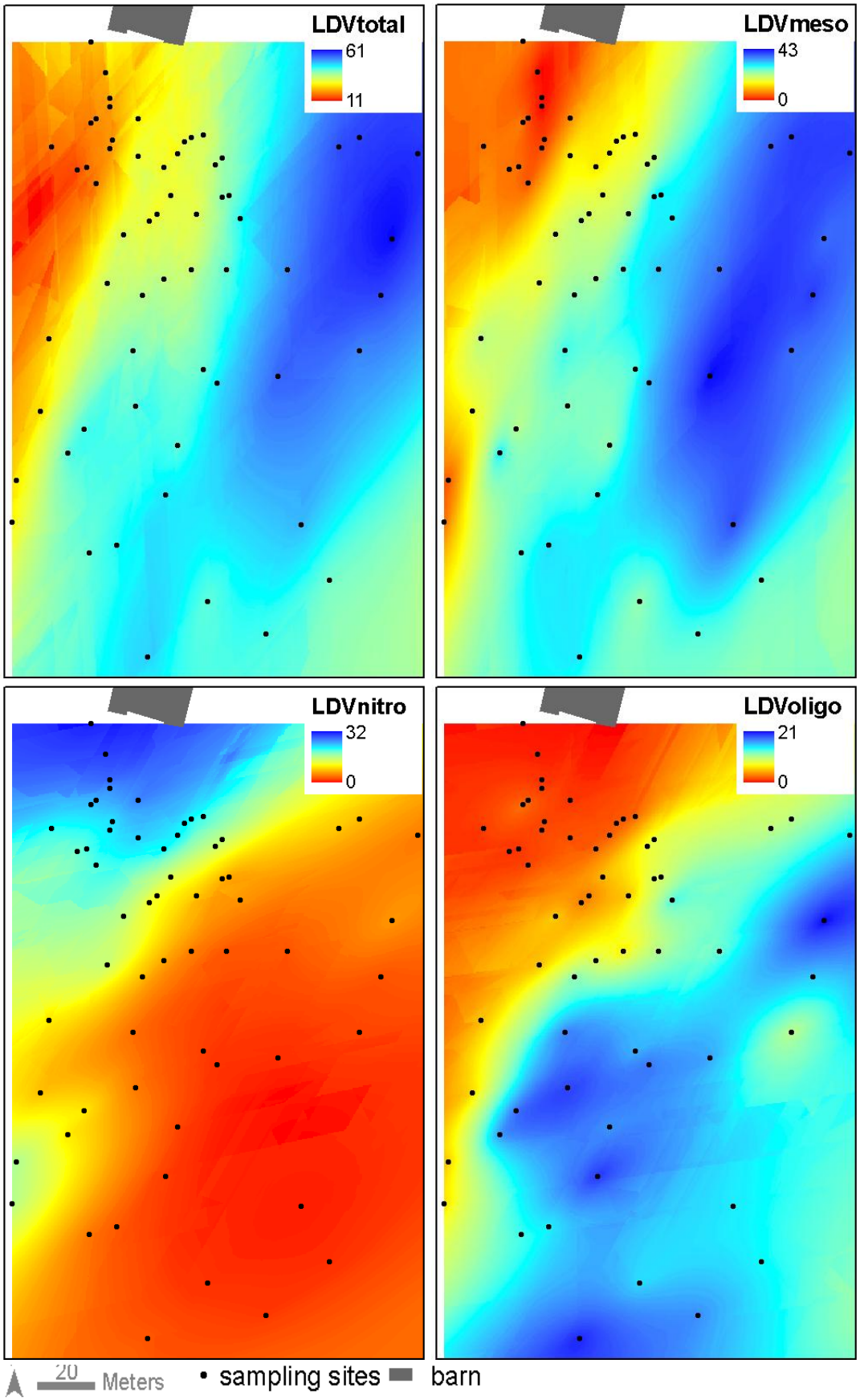

Figure 5: Interpolation of lichen functional-diversity variables, presenting total values (top left) and divided accordingly to functional traits regarding eutrophication tolerance. Black dots are the sampling sites (trees) the grey area is the barn.

The accuracy of the eutrophication-index used (Nimis and Martellos, 2008) was verified by correlating the species classification in the index with the respective PCA score, which was found to be highly significant, (Spearman R= -0.75, P=0.000029; N=24).

The correlation between the site scores of the PCA, factor 1 (Fig. 3), with the average NH_3 concentration (Fig. 4) on the same sites showed a very significant correlation where the positive portion of the first axis of the PCA was associated to lower NH_3 concentrations and the negative side to higher ones.

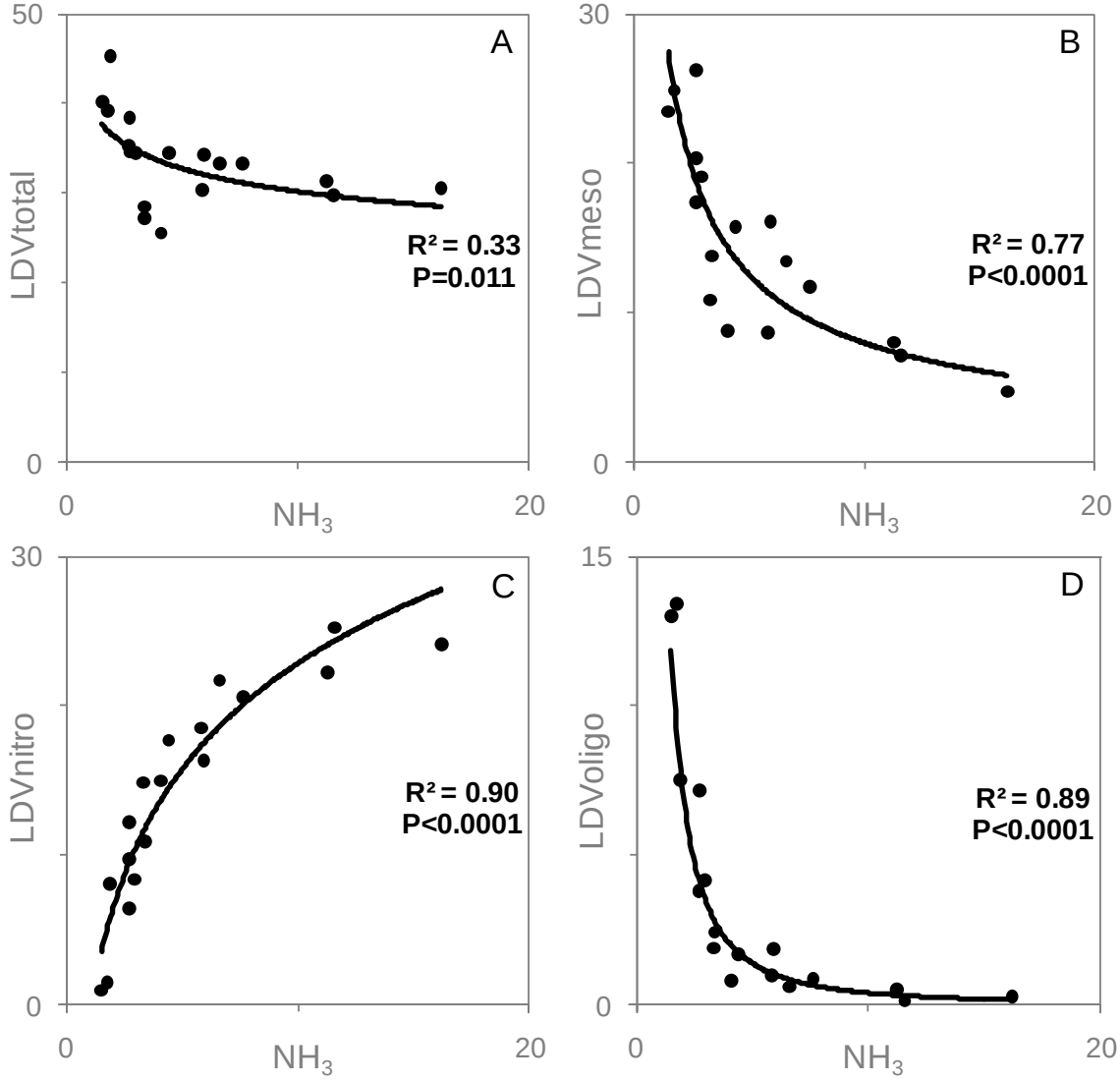

Figure 6: Relation between average atmospheric (NH_3) concentrations measured for one year period and four lichen functional-diversity variables: **A** all species (LDVtotal) and divided accordingly to eutrophication tolerance, **B** (LDVmeso: mesophytic), **C** (LDVnitro, nitrophytic) and **D** (LDVoligo, oligotrophic). Atmospheric NH_3 concentrations in $\mu g\ m^{-3}$. N=18.

Correlating NH_3 and lichen functional-diversity variables

The estimated values of the lichen functional-diversity variables were determined in the locations where the NH_3 was measured (Fig. 1) and were plotted against the annual average of atmospheric NH_3. For total LDV this correlation resulted in a weak logarithmic relation (R^2=0.29), with higher total LDV at the lowest NH_3 concentration (Fig. 6). However, when considering LDV divided accordingly to the corrected eutrophication-tolerance index (Nimis and Martellos, 2008), highly significant relations with NH_3 concentration were found (Fig. 6). The variables LDVmeso and LDVoligo were significantly and negatively related to NH_3 concentration. The variable LDVnitro was significantly and positively related with annual NH_3 concentration (Fig. 6)

DISCUSSION

The spatial and temporal atmospheric ammonia gradient

Both the average values and the spatial patterns of atmospheric NH_3 concentrations found in this work agree with the values found in other works made around NH_3 point-sources (Frati et al., 2007; Sutton et al., 1998). The highest values found here were registered near the source and then an exponential decrease for increasing distances from the cow-barn was found (Fig. 2, right). However a different temporal pattern has been observed, with NH_3 concentration presenting the highest values during autumn (Fig. 2, left).. In fact NH_3 emission are usually higher during the warmer season, which was seen both for natural sources (Blackall et al., 2008), agriculture point-sources (Harper et al., 2004) and multiple sources of NH_3 at a landscape scale (Dammgen, 2007). Higher concentrations in summer are justified in literature with higher temperatures (Harper et al., 2004; Huber and Kreutzer, 2002; Robarge et al., 2002; Sharma et al., 2010). However, all these studies were done in temperate climate. The effect of Mediterranean climate in NH_3 emissions is not yet well studied. The few studies performed in Mediterranean area were not designed to follow the seasonal pattern (Frati et al., 2008; Frati et al., 2006; Frati et al., 2007). Since Mediterranean summer is very dry, which was confirmed during this study (Fig. 1, right), there is very low soil water availability during this season, which has been shown in other occasions to reduce microbiological activity (Sommer et al., 2003) and reduce atmospheric NH_3 emissions. Moreover, the period on which higher NH_3 emissions were observed, mostly in the autumn (Fig. 2) coincide with periods with more rain and mild temperatures (Fig. 1). Supporting this hypothesis a work performed under monsoon climate has shown similar trends to the ones observed on our study, with higher NH_3 concentrations observed during the monsoon season (wet season) and lowest during the rest of the year (Kumar et al., 2004). Authors suggested that, due to a larger water availability the conditions during monsoon were more favourable for microbial activity (Kumar et al., 2004). However periods with no rain but high NH_3 emissions occurred (3-Aug. to 12 Sep., Fig. 1,2left), highlighting that the mechanisms that rules the NH_3 emission in Mediterranean areas are complex and deserve further study.

Testing the expert-knowledge classification on eutrophication tolerance

For the first time we have been able to test the classification of lichen species regarding eutrophication tolerance (Nimis and Martellos, 2008) using a single source of atmospheric NH_3. In general the classification performed well, what could be confirmed by: i) the very significant correlations between the distribution of species in the PCA and the NH_3 concentration measured (Fig. 4); and ii) the very significant correlation between the species classification on the eutrophication index and the species position on the first factor of the PCA. Nevertheless, of the 28 species tested, two were shown to be clearly misclassified, *Lecanora albella* and *Chrysothrix candelaris* (Fig. 3). These species, previous considered oligotrophic, should be reclassified as mesotrophic (1-3). In a work done in

Scotland Atlantic woods (Mitchell et al., 2005) it was found that, within a very low atmospheric NH_3 gradient (highest value of 0.2 $\mu g\ m^{-3}$) the species *Chrysothrix candelaris* was associated to the highest concentration of NH_3. Although it must be highlighted that the NH_3 concentration are much lower than the ones found here, there is at least a tendency of this species to tolerate some level of NH_3. This result confirmed that the eutrophication-index, which was developed based in the classification of species for Italy, is likely to perform well in Portugal and support the use of this index, corrected by the two species, for further works. Nevertheless, more tests of other species and in different climates against single sources of NH_3 should be made to further support the universal use of this functional diversity index.

Changes in lichen communities under an atmospheric ammonia gradient

It is well known that LDVtotal, or some other overall measure of lichen communities such as total species richness, is a sensitive ecological indicator, and can be used to monitor the impact of atmospheric pollution (Giordani, 2007; Pinho et al., 2004; Pinho et al., 2008a; Svoboda, 2007). However, these overall lichen-variables are only efficient when the pollution type present is detrimental for all species, such as in the case of e.g. SO_2 (Hawksworth and Rose, 1970) or atmospheric Cu (Branquinho et al., 1997). However, that is not the case of NH_3 that is a source of nitrogen, a nutrient essential to lichens (Honegger, 2009). Therefore it was not surprising that LDVtotal has only changed slightly with NH_3 concentrations presented the less significant correlations with NH3 it (P=0.011) (Fig. 6A) and showed that more than half (54%) of its total variance did not presented a spatial structure (Table 1). Other authors, depending on the intensity of the NH_3 gradient even found non-significant relations between lichen communities and NH_3 when using all species together (Frati et al., 2007; Van Dobben and Ter Braak, 1998).

The small response of LDVtotal to NH_3 concentration was due, in our case, to the opposing responses of species groups to NH_3: some species responded positively but others negatively (Fig. 4, 6). Thus, using total lichen diversity measures with NH3 gradients can mask the changes or the impacts in these communities.

Plant functional traits regarding tolerance to eutrophication are accepted as one of the explanations for species loss under increasing nitrogen availability (Suding et al., 2005). In fact, grouping lichens into functional groups, after correcting the two misclassified lichen species, has shown a clear and complete community shift from oligotrophic to nitrophytic species (Fig. 5, 6). This was supported by: i) the very significant correlations between species distribution on a PCA with the atmospheric NH_3 concentration (Fig. 4); and, ii) the very significant correlation between lichen functional groups and the atmospheric NH_3 gradient (Fig. 6, C, D). Other authors have also verified that the functional groups based on eutrophication tolerance (Nimis and Martellos, 2008) were related to the NH_3 concentrations, but only for Italy and for nitrophytic functional group (Frati et al., 2007). Here we have shown a good correlation for the entire community. Thus, for the first time here we show that the theoretical model of Sparrius (2007) is suported by experimental data (Fig. 6): oligotrophic species decline under increasing NH_3 concentrations and nitrophytic ones increase (Fig. 6 C, D). This result supports the use of lichen functional-groups as ecological indicators of atmospheric NH_3 effects in ecosystems.

Functional diversity as an ecological indicator of atmospheric NH_3 effects

Lichen functional groups have been used as indicators of nitrogen pollution both at a regional (Fenn et al., 2007; Rogers et al., 2009) and local scale (Frati et al., 2008; Ruisi et al., 2005). However in most cases only an increase in nitrophytic species could be related to an increase in NH_3. Moreover in those studies other environmental factors contributed to the observed change. In this area, where other environmental factors are either absent or their effects were homogenous, it was possible to detect

a clear and unique indication of the impact of NH_3 concentration on lichen functional diversity. Thus we have a high confidence for using lichen functional diversity as accurate ecological indicators of the effect of NH_3.

We used variogram analysis to evaluate the robustness of the lichen functional-diversity as indicators of the effect of NH_3. We showed that functional-diversity variables presented a spatial structure in most of their variance, in contrast with LDVtotal (Table 1). These results imply that two nearby trees are more likely to present similar values of LDVoligo than of LDVtotal. Taken together with the very significant correlations obtained between functional-diversity variables and NH_3 (Fig. 6), these results reinforce the use of functional diversity as accurate and robust indicator of the effects of NH_3.

CONCLUSIONS

The lichen functional-diversity based in eutrophication tolerance allowed us to observe that under increasing atmospheric NH_3 concentrations (Fig. 2) there is a complete community shift, with oligotrophic species being replaced by nitrophytic ones (Fig. 6 C,D), while total abundance is only slightly changed (Fig. 6 A). Moreover lichen functional-diversity variables LDVoligo and LDVnitro presented a high accuracy (Fig. 4) and robustness (Fig. 3, Table 1) as indicators of the effects of atmospheric NH_3. There is a broad potential of applicability, because these variables were calculated using standard methods (Asta et al., 2002) and the functional groups were defined accordingly to an international classification (Nimis and Martellos, 2008). Among these applications is the possibility to map with high spatial resolution the effects of NH_3 on ecosystems (Fig. 3) namely the critical level (Cape et al., 2009a). This could help to determine the effect of ameliorating policies, including abatement policies, strategies of ammonia sequestration or planting tree belts near cattle farms (Dragosits et al., 2006), nitrogen-related land-restoration policies such as land retirement (Fraser and Stevens, 2008) and determination of a safety distance between ammonia source and protected ecosystems. Moreover, lichens are among the most sensitive communities to NH_3 thus they could also be used as early-warning indicators of the effects of NH_3 for other not so sensitive components of the ecosystem. It is important to highlight that although lichen species are not the same all over the word, the functional groups are present, and can thus be applied everywhere.

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chapter 6

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06 | Assessment of critical levels of atmospheric ammonia for lichen diversity in cork-oak woodland, Portugal

INTRODUCTION

The effect of atmospheric ammonia on ecosystems has been the subject of ongoing research. Its adverse effects as an air pollutant are well characterised, and may be even more widespread than previously thought (Aber et al., 2003; Erisman et al., 2003; Krupa, 2003; Purvis et al., 2003). The most important sources of NH_3 in Europe are agricultural activities, mainly crop fertilization and cattle management (EPER, 2004; Galloway et al., 2003). Livestock housing facilities are recognised to be large point sources of NH_3 emissions. Close to such facilities, atmospheric NH_3 concentrations are very high, decreasing rapidly with distance over a few hundreds of meters to a few kilometres (Sutton et al., 1998). Measurements of atmospheric NH_3 in the vicinity of livestock housing reported values in Scotland of 24 to 59 $\mu g\ m^{-3}$ close to source, which declined to background values of 1.6 to 5 $\mu g\ m^{-3}$ at 1 km (Pitcairn et al., 2003; Pitcairn et al., 1998).

In order to assess the range of effects of NH_3 in natural ecosystems, that can be used for effective NH_3 mitigation policies (Dragosits et al., 2006), one can rely on two distinct approaches: i) direct measurements of atmospheric NH_3 concentrations, which provide an estimate of dry NH_3-N deposition, but require intensive and costly operations; ii) monitoring of effects on the biotic component. The latter approach should be carried out using groups of biota that are more sensitive to the pollutant of interest. Lichens have been reported as the most sensitive group to NH_3 emissions (van Herk, 1999; Wolseley et al., 2006a).

Lichens are symbiotic organisms widely used as biomonitors of environmental changes (Geebelen and Hoffmann, 2001; Geiser and Neitlich, 2007; Giordani et al., 2002; Nimis et al., 1991; Pirintsos and Loppi, 2003; Vokou et al., 1999). Monitoring atmospheric pollutants using lichens may be undertaken in three ways: 1) measuring variations in lichens diversity and/or abundance, 2) using variations in physiological parameters, and/or using lichens as accumulators of pollutants (Branquinho, 2001), and 3) considering functional groups related to nutrients tolerance, such as the division between nitrophytic/oligotrophic (or nitrophytic/acidophitic) groups (Ruisi et al., 2005; van Dobben and ter Braak, 1999; Wolseley et al., 2006b). For the Mediterranean region, a classification of lichen species based on their tolerance to environmental factors, namely eutrophication, is available (Nimis and Martellos, 2008), and was used for classifying lichen species in Portugal, under a similar climate.

The mechanism by which NH_3 affects lichens is under debate. It has been suggested that changes in substrata pH play an important role (van Herk, 2001), a mechanism that may be confounded by the effects of dust, especially in Mediterranean regions (Loppi and Pirintsos, 2000).

In this work our objective was to establish the critical levels of atmospheric NH_3 based on changes in epiphytic lichen communities in a Mediterranean climate.

MATERIALS AND METHODS

Study area

The study area is a farm (called Malhada-de-Meias) with a cattle-breeding site located in Portugal, 30 km East of Lisbon (Fig. 1c). Soils at the farm are sandy alluvium and eolic deposit, the average annual temperature is 17.5 ºC and the average annual precipitation is 600 mm (averages 1931-1960) (IA, 2006). The farm is surrounded by intensive agriculture and livestock activities, and about 200 cows are permanently housed in a single barn measuring 800 m^2 (Fig. 1). To the south of this barn lies a cork-oak woodland (*Quercus suber L.*) grazed by cattle. Some areas of temporary pasture

are also present in the woodland that is mainly for sheep. Ten kilometres west from the farm, a main motorway and urban areas are also located. The study area presents a Mediterranean climate (Blondel and Aronson, 1999; Thompson, 2005), in the thermomediterranean climatic belt (Rivas-Martinez and Rivas-Saenz, 2009), characterized by dry hot summers and rainy mild winters (see climatogram in Fig. 1).

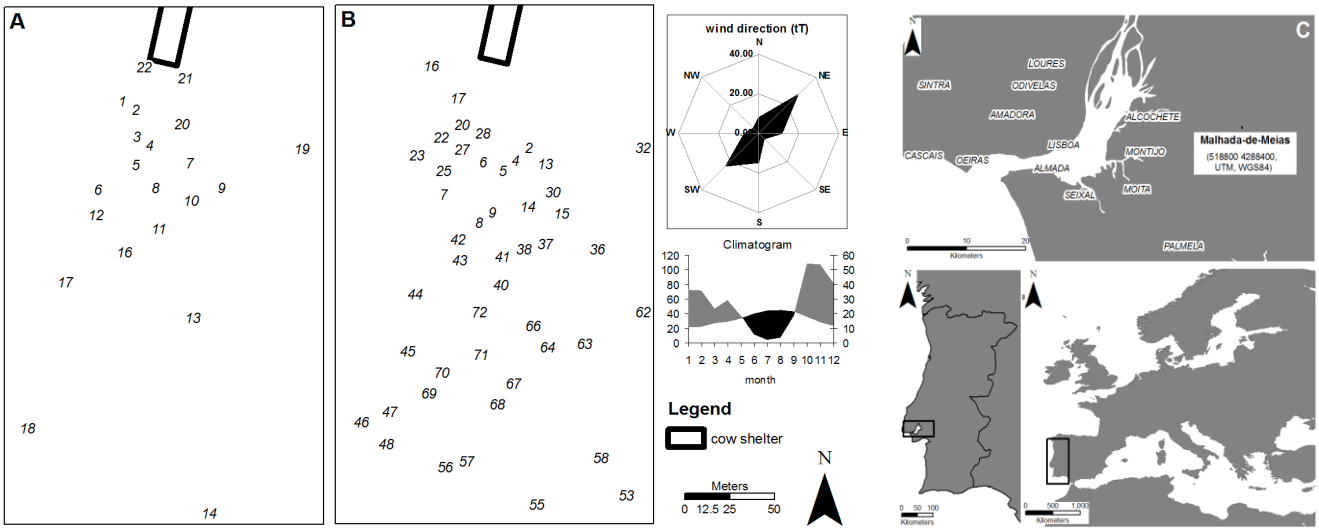

Figure 1: A: location of the alpha sampling point (n = 22; site 15 is located 800m to the SE, and is not shown), B: location of the lichen diversity sampling plots (n = 74 trees). Wind direction represents the % of hours in which wind blows from the prevailing direction, during the study period. The climatogram refers to the average monthly precipitation (left) and average monthly temperature (right), from 1985 to 2006, the black area represents the xeric period according to Gausen, 1954 (in Blondel and Aronson, 2004): C: General location of the study area showing main cities and the location of the study site.

Ammonia sampling

Atmospheric NH_3 concentrations were measured over three continuous sampling periods in autumn 2006 (2-weekly measurements starting 3-Oct, 24-Oct and 6-Nov) using the high sensitivity ALPHA (Adapted Low-cost Passive High Absorption) passive diffusion samplers (Tang et al., 2001). Measurements were carried out at 22 sampling locations (2 x ALPHA samplers at each point) downwind of the cattle barn (Fig. 1a), with a larger proportion of sites within 200 m of the barn, since that was where the largest variation in concentrations were expected. Ten field blanks were randomly distributed amongst the sampling points.

At four sampling locations, additional ALPHA samplers provided by CEH were set up in parallel to those prepared by University of Lisbon for quality assurance purposes. Ammonium concentrations collected on the filter papers in the ALPHA samplers were determined by a modification of the Berthelot reaction (Cruz and Martins-Loução, 2000). Results were compared with those obtained for the parallel CEH samples that were analyzed independently by CEH using the AMFIA (AMmonium Flow Injection Analysis) system (Sutton et al., 2001). Atmospheric ammonia concentrations determined with the ALPHA samplers were calibrated against measurements obtained with a CEH DELTA (DEnuder for Long-Term Atmospheric sampling) system. A good correlation was observed between the different methods used to determine atmospheric NH_3 concentration in the air. The results presented are those obtained with the ALPHA samplers.

Lichen diversity

Lichen diversity on the main trunks of cork oak trees was measured using a sampling grid accordingly a standard protocol (Asta et al., 2002; Scheidegger et al., 2002), but selecting trees differently. For each atmospheric NH_3 ammonia sampling point, the

nearest 3 or 4 trees (in total, 74 trees) were selected for a survey (Fig. 1b). For each tree, a 50 x 10 cm grid, divided into five 10 x 10 cm subunits was placed on the tree trunk above the cork-harvest zone and used to determine lichen species frequencies, in each of the four main aspects.

A lichen diversity value (LDV) was calculated for each atmospheric NH_3 sampling point (Asta et al., 2002). LDV accounts both for species number and frequency. We calculated total LDV (using all species) and also the LDV of functional groups regarding nutrient preferences, according to (Nimis and Martellos, 2008) and considered the highest classification for each lichen species. Groups were classified as: 4 and 5 as the nitrophytic group (LDVnitro), 1 and 2 as the oligotrophic group (LDVoligo), 5 as the strictly nitrophytic species group; and 3 as the intermediate group. Outliers were excluded from the analysis of critical levels.

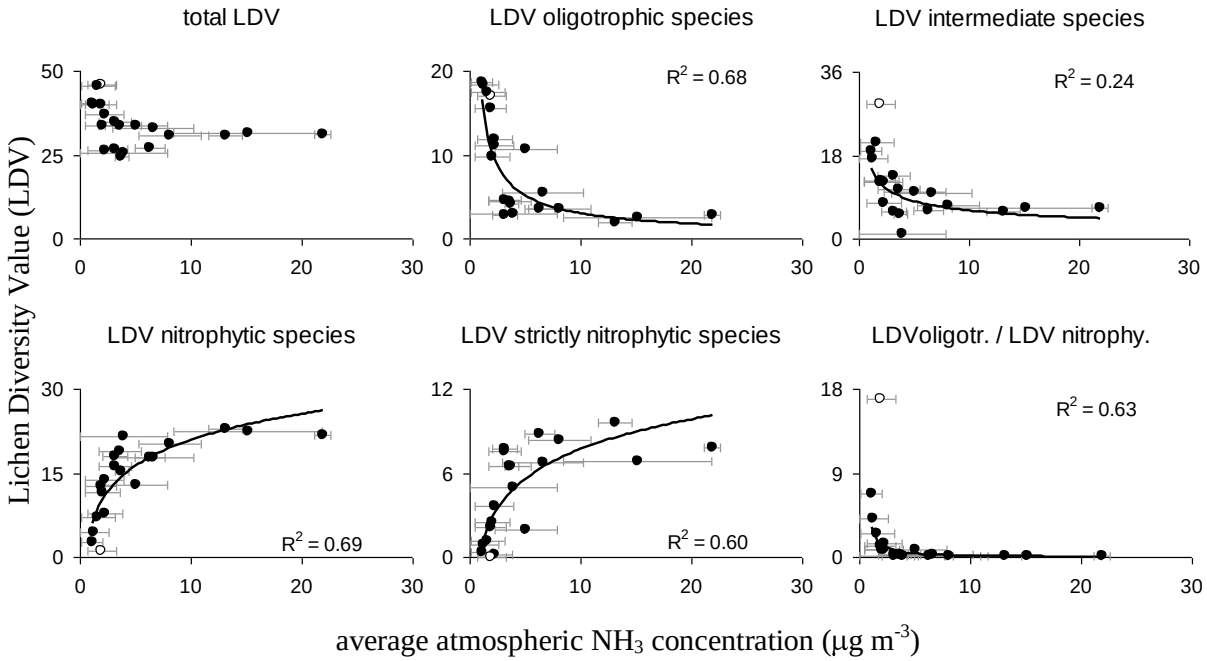

Figure 2: Relationship between several lichen diversity indicators and atmospheric NH_3 concentration. Fitted line is a log function for downward curves and power function to upward curves. Site 15 is represented by an empty circle. Error bars represent the standard deviation of NH_3 concentration measured during the 3 sampling periods and were not considered in the adjustment of the fitted line.

RESULTS AND DISCUSSION

Relating lichen diversity to atmospheric ammonia concentration

The relationship between several lichen diversity indicators and atmospheric NH_3 concentration is depicted in Fig. 2. In general, at greater atmospheric NH_3 concentrations, lower total LDV values were found, showing that NH_3 has a significant impact in lichen communities. However, by looking at LDV divided into different nutrient functional groups, a much clear pattern is observed: for increasing NH_3 concentrations the oligotrophic lichens species decreased logarithmically from 18 to 2, whereas nitrophytic lichen species increased from 1 to a maximum of 20. Besides the two functional group indicators, and because LDVnitro and LDVoligo have an opposing pattern with atmospheric NH_3 (Fig 2), the ratio between LDVoligo and LDVnitro was also plotted. It was found that this indicator showed a significant correlation with NH_3, reflecting at the same time the pattern of the two different functional groups.

This relationship between the studied indicators and NH_3 was further interpreted by looking to the temporal changes of the atmospheric NH_3 represented by the standard deviations. Due to the fact that only three measurement periods were available, it was useful to evaluate this variability. Greater temporal changes are probably due to short

term events, such as temporary pasture in a specific location, which temporally increase the NH_3 concentration but have a reduced effect in LDV. That is probably the case of sampling location 15, which experienced periodic pasture by sheep and consequently higher NH_3 concentrations, but which is characterized by a high LDVoligo and a very low LDV nitro. This sample was excluded from the critical levels analysis. Another explanation for the greater standard deviations may be the existence of contrasting periods in the prevailing wind direction. For further analysis, the three indicators LDVoligo, LDV nitro and LDVoligo / LDV nitro ratio were chosen, since they showed the best correlation with NH_3. Given that most biodiversity changes occurred up to 4.5 μg m^{-3}, we focused further analysis at that level. Most authors have also reported that changes in lichens communities occurred at this level (Wolseley et al., 2006a), detecting changes in lichen diversity for concentrations above 3 to 4 μg m^{-3}.

Mapping of lichen diversity and atmospheric ammonia concentration

The chosen indicators were interpolated using a simple interpolation function (inverse squared distance weighted) and mapped for the study area (Fig. 3). The observed pattern was a replacement of oligotrophic communities, more abundant at sites distant from the barn, by nitrophytic communities that become more abundant nearer the barn. This pattern is highlighted by the spatial distribution of NH_3 concentration that increases sharply near the barn, as predicted.

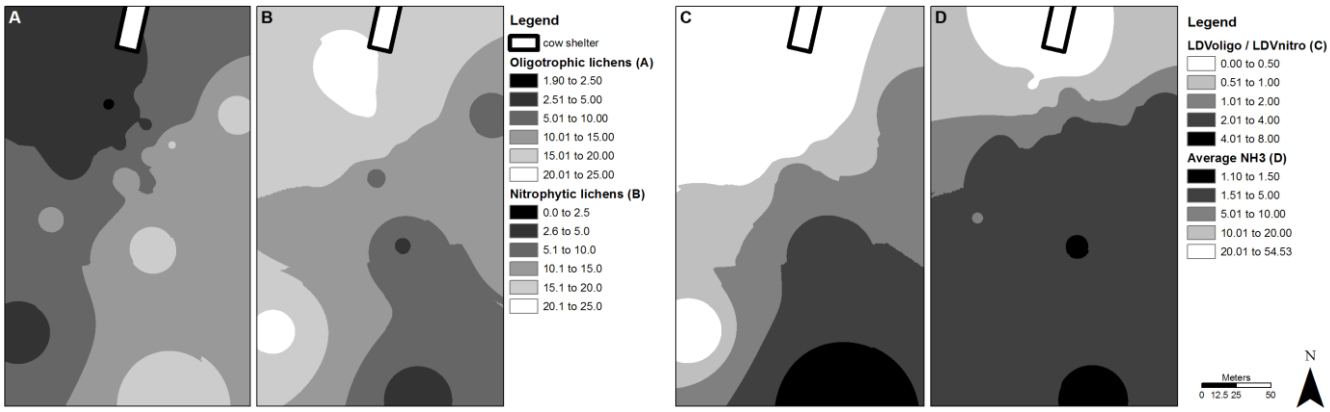

Figure 3: a) LDV of oligotrophic species (LDVoligo); b) LDV of nitrophytic species (LDVnitro); c) LDVoligo/LDVnitro ratio; d) average concentration of NH_3 (μg m^{-3}) for a six week period.

LDV levels in undisturbed sites

When defining critical levels for biodiversity, it must be assumed that any changes should be related to pristine and/or undisturbed sites. In this study, the NH_3 gradient was found always within the same farm, which has NH_3 sources other than the cattle barn, such as: temporary pastures, soil mobilization, manure application, etc. To avoid the problem of not having a clear undisturbed site, the lichen diversity indicators at the farm were compared with indicators found at two distant control sites in the region: one without any livestock and with very low forestry activities and the other without livestock but with some forestry activities, such as selective tree cutting and cork harvesting. These two control sites were located 5 km north of the study site, and biodiversity data was collected using the same methodology. The distribution for the selected LDV indicators (Fig. 4) was compared, which shows that the biodiversity gradient at the farm can be considered to be disturbed, presenting on average lower LDVoligo and higher LDV nitro. Nevertheless, the maximum and minimum values of all three sites were within the same range, in particular the control site with some forest activities (lower line in LDVoligo of Fig. 4) was similar to the more undisturbed sites in terms of NH_3 gradient.

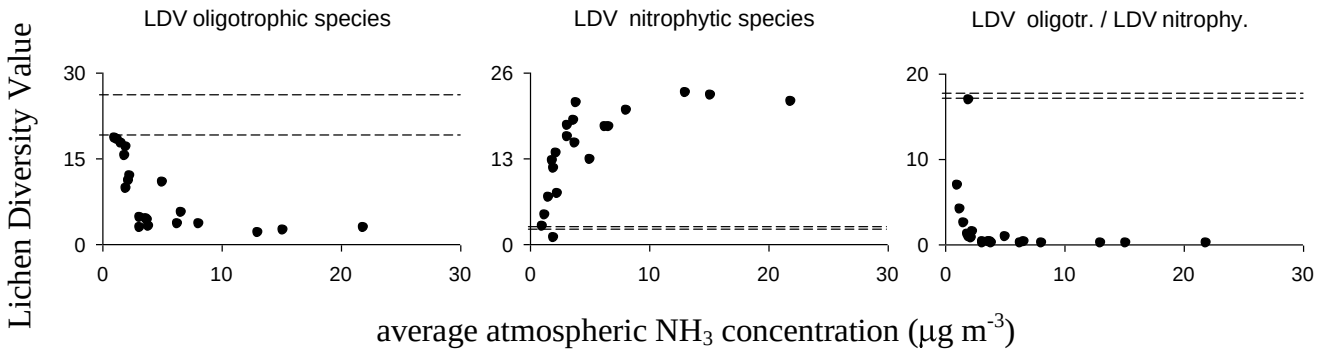

Figure 4: Comparison between the distribution of lichen diversity indicators (LDV) of the farm site (represented by black dots) and the average of two control sites (represented by the two lines), located 5 km away and for which the same methodology was followed

Detecting threshold levels

In order to determine the critical levels in atmospheric NH_3 concentration for lichen diversity, the focus was on concentrations up to 4.5 µg m^{-3}. It is in this range of concentrations that most changes in lichen diversity occur and where the pattern between the two variables is linear (Fig. 5.). In the case of LDVnitro, the control values found in undisturbed sites were very similar to the lowest value found on the farm (Fig. 5). A critical level was calculated for NH_3 using an estimate with a probability level of 95% between NH_3 and LDVnitro data fitted to a linear model (Fig. 5). Sampling points where the first visible changes in the LDVnitro occurred were used. This was found to be 1.7 µg m^{-3} for the LDVnitro indicator. In the case of LDVoligo, one of the controls calculated for undisturbed sites was much higher than the maximum level of this indicator, whereas the control with forest activities was within the expected range of the data. Using the methodology described above for LDVnitro, the critical level was determined as 1.4 µg m^{-3} for LDVoligo (Fig. 5). When applying the same methodology for the LDVoligo/LDVnitro ratio, a similar threshold was obtained (Fig. 5). Interestingly, these values show that oligotrophic lichens are slightly more sensitive than nitrophytic ones which is in accordance to findings by (Wolseley et al., 2006a). Surprisingly, the critical levels found here are very similar to values determined in other countries for the same organisms namely in the UK (Wolseley et al., 2006a), where a loss of lichen diversity (measured in classes of abundance) was observed at concentrations above 3 to 4 µg m^{-3}, but in Mediterranean climates that have higher temperatures during the summer, we would expect that lichen diversity critical levels would be different from the those reported in colder climates. The critical levels could be similar due to the fact that the same mechanism may be involved in establishing the changes in lichen communities, independent of climate. Atmospheric NH_3 is known to exhibit large seasonal variability in concentration (Sutton et al., 2001); the actual annual mean concentrations may therefore be either larger or smaller than the estimates from only 3 sets of measurements made over a 2 month period in autumn. An accurate assessment of the annual mean NH_3 concentrations would require continuous measurements for at least a year, which could then be used to provide a better estimate of critical levels for atmospheric NH_3.

Impact of atmospheric NH_3 in lichen communities and their use as biomonitors

It has been observed that with increasing air NH_3 concentrations, lichen communities changed from oligotrophic to nitrophytic species. The mechanism by which atmospheric NH_3 influences lichens is still under debate, but it has been suggested that both a direct impact and an indirect impact, by changing bark pH may occur (Frati et al., 2006; Loppi and Pirintsos, 2000; van Herk, 1999; van Herk, 2001; Wolseley et al., 2006a). Lichen diversity responds clearly to increases in NH_3 concentration. They are

also long-living organisms, able to integrate environmental changes over time. Therefore, lichen diversity is a suitable biomonitoring method, by providing an important measure of the direct cumulative dose-response to exposure to NH_3 emissions, which may vary rapidly over time (Jovan and McCune, 2006).

By examining changes in lichen communities, specifically by using lichen indicators based on nitrogen-tolerance, an estimate of atmospheric ammonia critical levels could be made for Mediterranean regions. This critical level, between 1 and 2 $\mu g\ m^{-3}$ was found to be lower than the actual limits ($8\mu g\ m^{-3}$) but is in accordance with the concentrations found in other works using lichens in this chapter Because critical levels seem to be similar in regions with contrasting climate, the actual mechanism involved in atmospheric NH_3 tolerance in lichens needs further study, as well an enlightenment of a possible role of climate in this sensitivity.

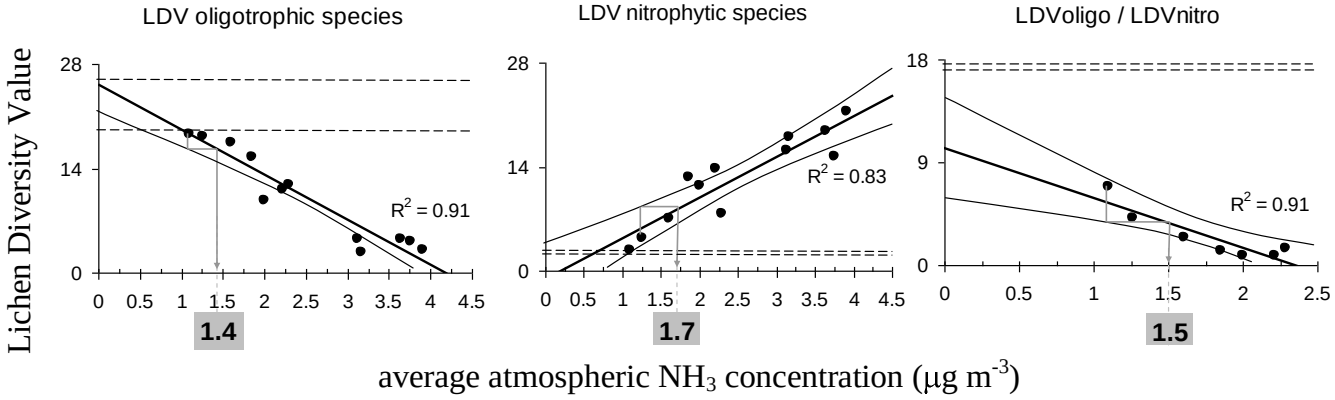

Figure 4: Focus on the linear gradient range, showing the relationship between three lichen diversity indicators and concentrations of atmospheric NH_3. The highlighted NH_3 concentrations are those for which changes in lichen diversity have occurred.

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chapter 7

This chapter is based on the following publications:

Pinho P, Martins-Loução MA, Máguas C, Branquinho C (2011) Calibrating total nitrogen concentration in lichens with reduced nitrogen emissions at regional scale. In: Nitrogen Deposition, Critical Loads and Biodiversity (Eds. Sutton M.A. et al.) (Proceedings of the INI/CLRTAP/CBD Expert Workshop, 16-18th November 2009) (in press).

Pinho P, Máguas C, Cruz C, Martins-Loução MA, Branquinho C (in press) Selecting critical areas for monitoring the impact of ammonia on biodiversity. Springer.

07 | Calibrating total nitrogen concentration in lichens with emissions of reduced nitrogen at the regional scale and its application for conservation

ABSTRACT

The impact of nitrogen (N) on ecosystem functioning and biodiversity is currently recognized to be increasing worldwide. For that reason, there is an urgent need of strategies aimed at identifying and mitigating N mediated effects. Most studies undertaken at a regional scale regarding N deposition are based on models. However, there is a missing link between the predictions made by N deposition models at broad spatial scales, and the actual atmospheric N deposition. Our aim was to use biomonitors to provide that link. More specifically our objective was to present evidence that N concentrations measured in lichen thalli can be used as an ecological tool to assess the deposition of atmospheric reduced N in ecosystems. To do so we have related N concentrations measured in lichens thalli to ammonia emissions estimated from cattle numbers and to the cover of agricultural land. This was done in two areas with a Mediterranean climate in south-west Portugal. The results have shown that N concentrations in lichens could be significantly correlated with reduced N emissions from regional sources. This lichen variable can thus be used as an ecological tool to map, with high resolution and at a regional scale, N deposition in ecosystems. This lichen variable may help identify areas of high N deposition where further monitoring may be required to help safe-guard against Critical Load exceedance and Biodiversity impacts. Knowing this, we used N concentration in lichens as an ecological indicator and for conservation purposes mapped the areas under greater N deposition within a Natura 2000 site and two Special Protection areas. We could then select potential critical areas due to atmospheric N deposition, where further studies should now be focused.

INTRODUCTION

Pollution by nitrogen (N) was recently recognized not only as a major threat to biodiversity and ecosystem functioning but also a threat that is expected to increase worldwide (SCBD, 2006). Moreover, together with biodiversity loss and climate change, the alteration in the N-cycle is considered one of the three global drivers whose changes have already passed the threshold beyond which unacceptable environmental change could occur (Rockstrom et al., 2009). In fact, the negative impact of N on ecosystem functioning and biodiversity has been confirmed in numerous works (Aber et al., 2003; Bobbink et al., 2010; Erisman et al., 2003; Kleijn et al., 2009; Krupa, 2003; Phoenix et al., 2006; Purvis et al., 2003; Suding et al., 2005).

The main anthropogenic sources of reduced N, mainly atmospheric ammonia (NH_3), are agricultural activities, mainly intensive farming applying fertilizers and intensive animal husbandry (EPER, 2004; Galloway et al., 2003). The studies dealing with the deposition of reduced N at a regional scale mostly rely on data derived from models (Phoenix et al., 2006). However, because models work at very broad spatial scales and the effects, particularly those related to NH_3, are very local (Pinho et al., 2009), there is often a mismatch between modelled deposition and actual deposition at the local scale. To improve our understanding of the effects of deposition we need more reliable means of quantifying it in the absence of effective measuring networks (Phoenix et al., 2006).

Lichens can be use to validate the predictions made by models to the actual N deposition on ecosystems. Lichens are organisms with particular physiological characteristics that make them well suited to acting as biomonitors. These characteristics include the absence of a cuticle and

roots (Honegger, 2009), allowing lichens to take up water and dissolved nutrients directly from the atmosphere. Lichens have been shown to accumulate a large number of pollutants (Augusto et al., 2010; Augusto et al., 2004; Branquinho et al., 1999; Branquinho et al., 2008). However, N is a special case being a macro-element essential for life. Despite this, some authors have shown that N can be accumulated in relation to N deposition (Branquinho et al., in press; Gaio-Oliveira et al., 2001). For this reason, the N concentration in lichens has been used in local-scale studies dealing with N impact or deposition. However, these studies were mostly undertaken close to point sources such as cattle barns (Branquinho et al., in press; Frati et al., 2007; Olsen et al., 2010). Here we have assessed the relationship between N concentrations measured in lichens and N emissions at a regional scale.

Our objective was to provide evidence to support the use of N concentrations in lichens as an ecological tool to assess, at a regional scale, the deposition of atmospheric reduced N. Specifically we wanted to address the following questions: (i) are N concentrations in lichens related to potential sources of reduced N at a regional scale? (ii) Can N concentration in lichens be used to estimate NH_3 emissions with high spatial resolution? (iii) Can we apply this knowledge to evaluate the potential impact of atmospheric reduced N deposition on areas of high conservation interest such as the Natura 2000 network?

METHODS

This study was carried out in a region with a Mediterranean climate located in south-west Portugal (Fig 1). The region has diverse land-use types including urban, industrial, agriculture and semi-natural vegetation as well as a large number of Natura 2000 sites (Fig. 1). Two large areas were studied in which the N concentrations in lichens growing at a number of sampling sites were determined. Sampling sites were distributed in the two areas at an average minimum distance of 3360m of each other. Selection of sampling sites avoided direct pollution sources, in order to reflect background conditions. In each area we also estimated potential NH_3 emissions using two approaches: i) land-cover information in order to estimate the N emissions from all agriculture activities; and ii) animal census data in order to estimate N emissions from cattle only.

Using Corine LandCover 2000 (Caetano et al., 2009) the area occupied by agriculture in the neighbourhood of each sampling site was determined (Pinho et al., 2008a). Several agricultural types were considered: i) permanent agriculture - orchards and olive groves (Corine class 22); ii) heterogeneous agriculture - small farms with animals and vegetables (Corine class 24); and iii) annual agriculture - large areas with grain cultures (Corine class 21). N emissions from cattle were estimated from agricultural census's at a civil-parish level (INE, 1999), the most detailed level available. All types of cattle were considered in the calculations, using NH_3 emission factors available for each animal type (IPCC, 2006). Total annual values were then divided by civil-parish area. Only civil-parishes containing one or more sampling sites were included in the correlation analysis. N concentration was measured in the lichen thalli of *Parmotrema hypoleucinum* (J.Steiner) Hale (south area, 71 sites) and *Xanthoria parietina* (L.) Th.Fr. (north area, 64 sites): 3 determinations from a composite sample of 15g.

For the correlation analysis, the N concentration in the lichens was used in two different ways: Firstly, for correlating with neighbouring land-cover, each sampling site was considered independently and correlated to the area occupied by agriculture. This was done in a spatially explicit way using moving correlation analysis (Pinho et al., 2008b). Secondly, the lichen N concentration values were interpolated within each of the two study areas using ordinary kriging after variogram analysis, and the average value for each civil-parish was determined. This average value for each civil-parish was then correlated with the NH_3 emissions estimated from cattle data. Using the interpolated values derived from kriging ensured the average values were very robust regarding any distortion on the spatial

distribution of sampling sites within each civil-parish, ensuring that the parish lichen-N values were representative of the entire parish. Finally a detailed analysis of the map of N concentration in lichens was made for a smaller area where three important Nature conservation areas can be found: a Natura 2000 site "Comporta/Galé", and two Special Protection Areas (Birds Directive), "Lagoa de Santo André" and "Lagoa da Sancha".

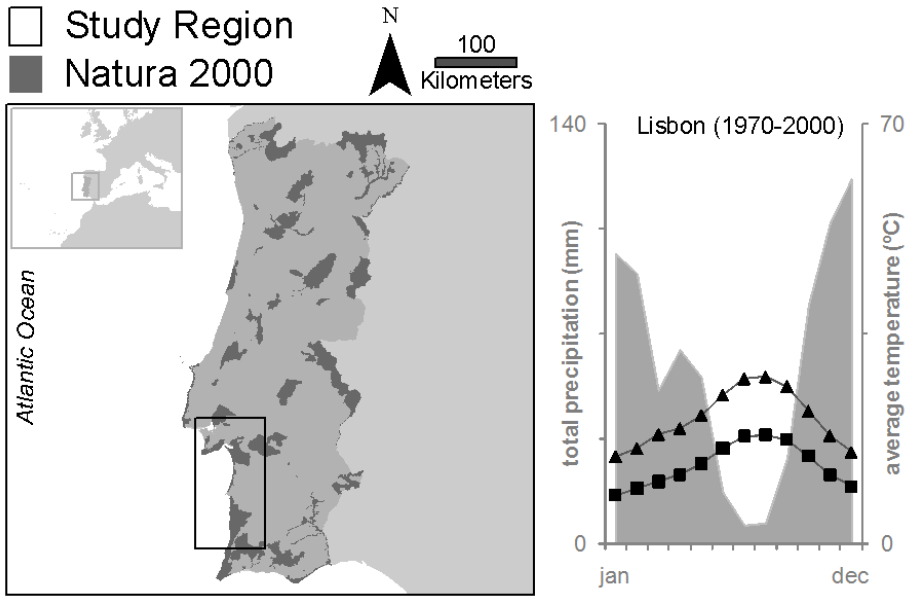

Fig. 1: Location and climatic characterization of the studied region. The Natura 2000 sites in mainland Portugal are also shown. In the climatogram the lower axis corresponds to months (from January to December), left axis average monthly total precipitation (mm) represented by the filled shape, right axis monthly temperature (ºC) using averages of the maximums (triangles) and minimums (squares). Values are averages from 1971 to 2000 (IM, 2000).

RESULTS

The mapping of N concentration in lichens has shown that this variable had some spatial continuity (Fig. 2, top). Several hotspots of higher concentration were detected for both studied areas. Land cover mapping (Fig. 2, bottom) revealed that the region represents a typical Mediterranean landscape, with semi natural-land-cover mixed with agriculture and scattered urban areas. Mapping of NH_3-N emissions from cattle (Fig. 2, bottom) has shown a large emission gradient, including rather high values (~80 kg N ha^{-1} $year^{-1}$). The highest emissions occurred in the northern civil-parishes due to higher cattle densities.

Using local correlation analysis (Pinho et al., 2008b) a large number of significant and positive correlations were found between N concentration in lichens and the neighbourhood area occupied by agriculture (Fig. 4). The higher the areas occupied by annual and by heterogeneous agriculture, the higher the N concentration measured in lichens.

Significant and positive correlations were also found between NH_3 emissions estimated from cattle and N in lichens (Fig. 4). The civil-parishes with higher N emission from cattle presented the highest average N concentration measured in lichens. This was found for both studied areas.

A more detailed analysis on an area located on the southern coast of the studied region (Fig. 3), has shown a detailed spatial pattern of N concentrations, including one hotspot of higher N concentration located within the nature protection areas (Fig. 5).

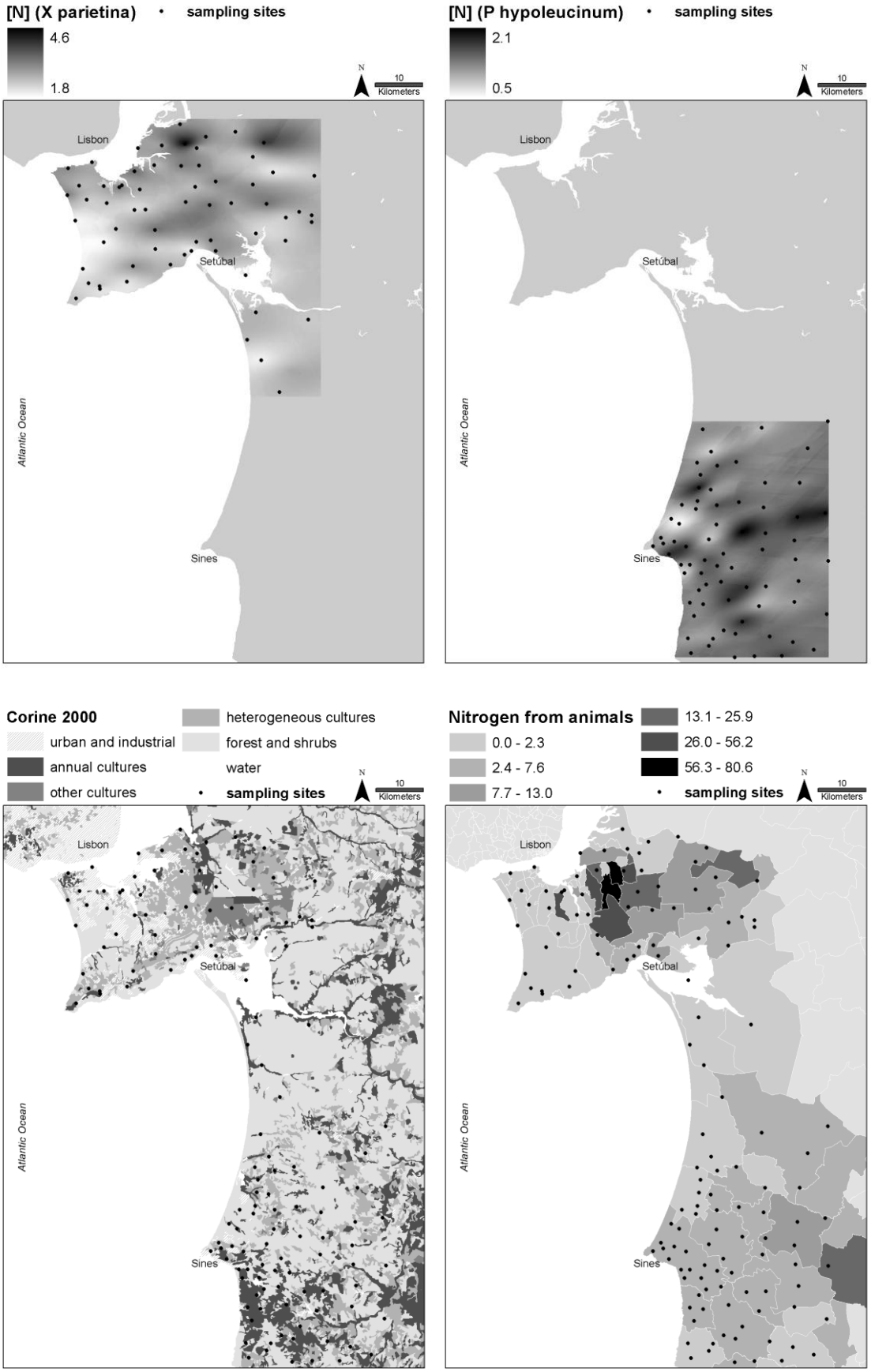

Fig 2: Top: mapping of N concentrations measured in lichens, in % (N) for *Xanthoria parietina* (left) and *Parmotrema hypoleucinum* (right). Bottom: Corine Land Cover 2000 map (left) and NH_3 emissions from cattle estimated from agriculture census at a civil-parish level, in kg $ha^{-1}y^{-1}$ (right).

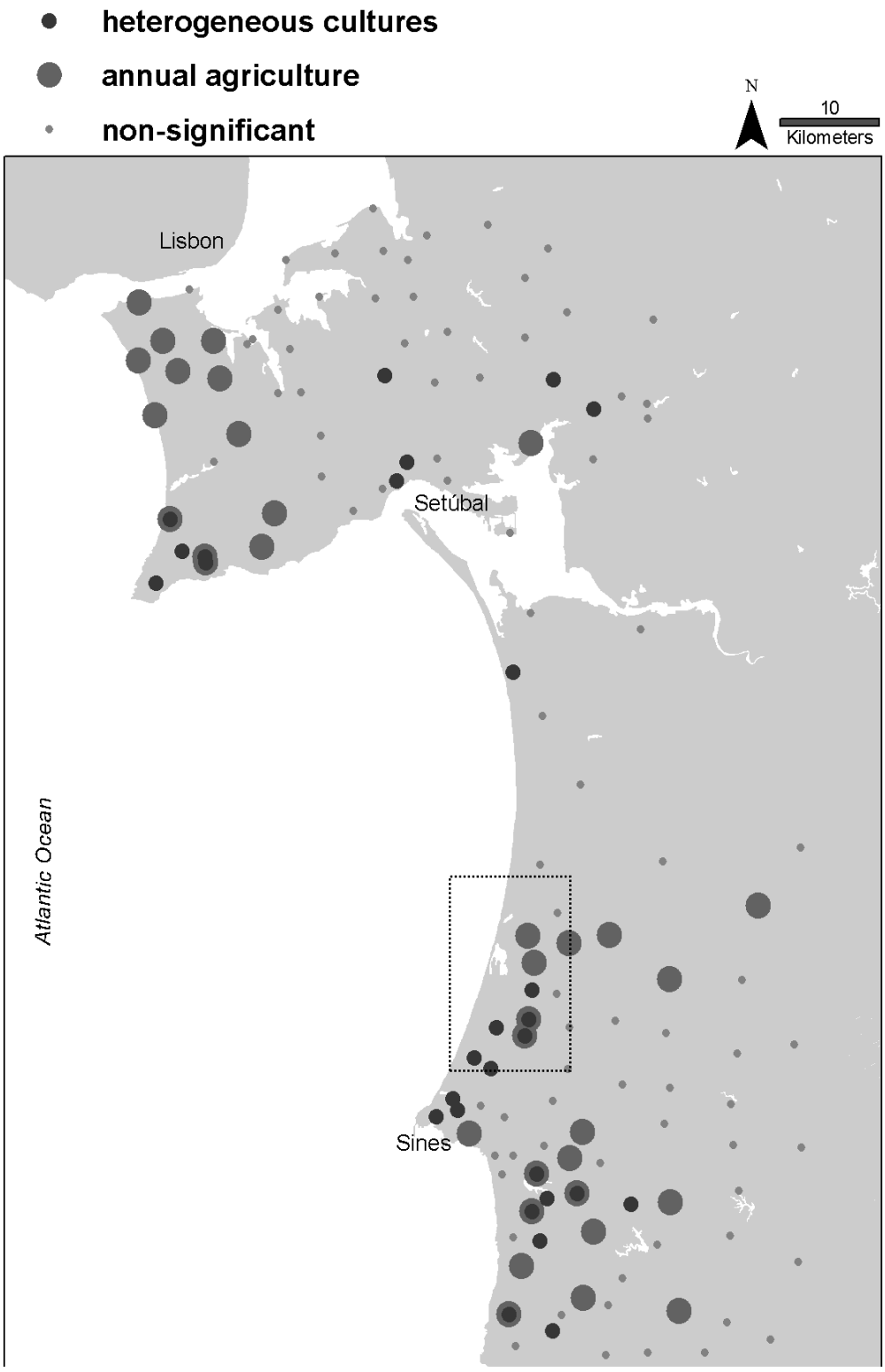

Fig. 3: Mapping of a correlation analysis between N concentrations in lichens and neighbouring land-cover. This is the result of a moving window analysis that correlates two variables, using as samples all sites at a distance of 10 km from each sampling site (Pinho et al., 2008b). A significant correlation indicates that, within a 10 km radius neighbourhood, the two variables are significantly correlated. In the maps, non-significant correlations ($P>0.05$) are marked with small dots, significant ones with larger circles. The two regions were correlated separately (because N concentration was determined in two different lichens species) but were plotted together. Land-cover was determined from Corine Land Cover 2000 and a number of neighbouring distances were tested, the one with the highest correlation was plotted (Pinho et al., 2008b). All significant correlations were positive. The square shows the area where a detailed analysis of N concentration was made for conservation purposes (Fig. 5).

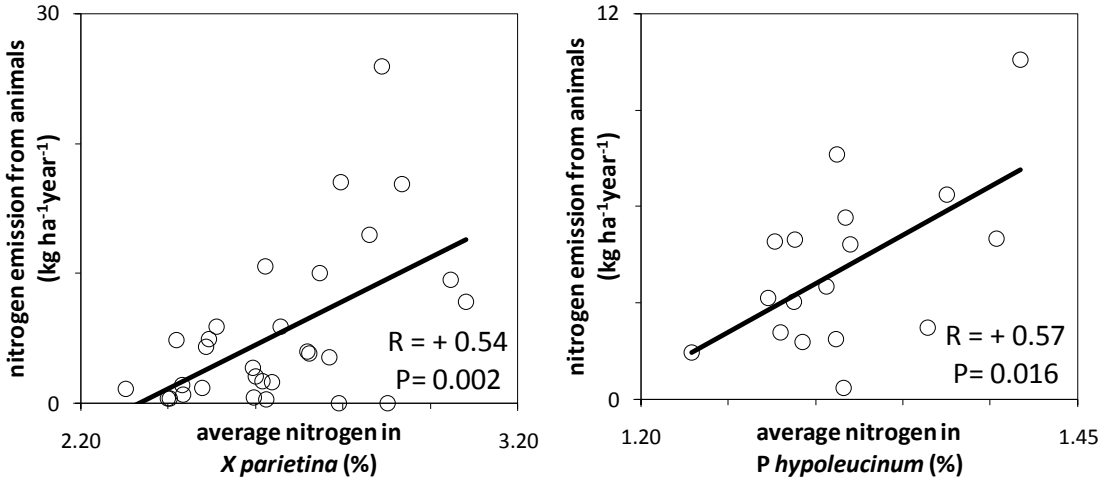

Fig. 4: Relationship between reduced N emissions calculated from cattle census data and N concentration in lichens, for the two studied areas. Lichens used were *Xanthoria parietina* (left) and *Parmotrema hypoleucinum* (right). The correlation was calculated for the civil-parish level using all parishes with at least one sampling site within and the average N concentration in lichens within that civil-parish. n=17 (left) and n=30 (right).

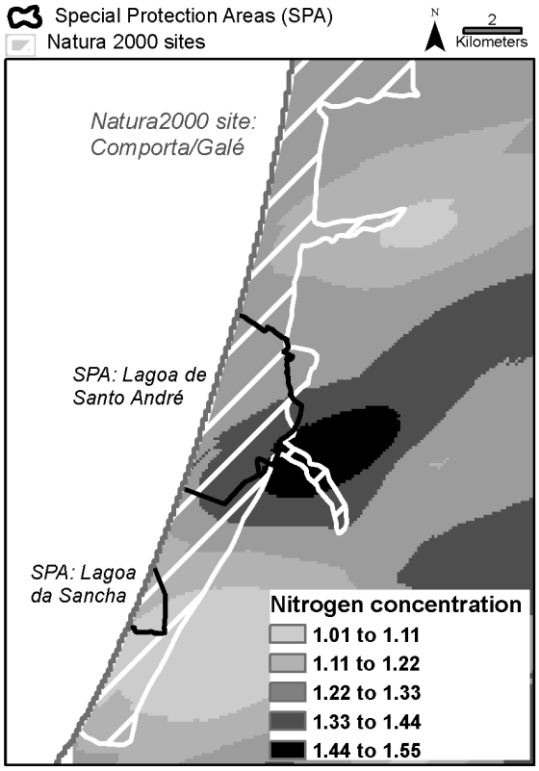

Figure 5: Mapping of the interpolation of N concentration (% dry weight) measured in *Parmotrema hypoleucinum* within the study area, on which one Natura 2000 site and two special protection areas are located.

DISCUSION

Nitrogen concentration values differed between the species with the highest values observed for the northern area in *Xanthoria parietina*. This species was expected to contain the highest concentration, because it is a nitrophytic species (Nimis and Martellos, 2008). Nitrophytic species are known to contain higher N concentrations than non-nitrophytic ones (Gaio-Oliveira et al., 2001). However, this difference did not influence the

correlation analysis because it was performed independently for each area.

The N-concentration observed for *Parmotrema hypoleucinum* in our study falls well within the N-concentration from literature: 0.5 and 2.1% (Palmqvist et al., 2002) and between 1.55 and 2.56% in *Flavoparmelia caperata* (Frati et al., 2007). However the N-concentration observed for *Xanthoria parietina* in our study was higher than the ones published: 1.34 to 3.34% (Gaio-Oliveira et al., 2001) and 1.66 to 3.35% (Frati et al., 2007). This probably reflects the fact that, unlike the cited studies, the lichens used in this work, were *in situ* lichens, with prolonged exposure to NH_3 deposition favouring N accumulation, leading to higher N concentrations. Another hypothesis may be related to the fact the studied areas had higher atmospheric N deposition than used in previous studies, and that lichens can reflect the higher deposition. Thus, lichen thalli N concentrations not only reflect NH_3-N deposition in a given local area, but also can be used as tools to study large gradients of environmental N deposition.

Were N concentrations in lichens related to potential N sources at a regional scale? Significant and positive correlations were found at a regional scale between the N concentrations in lichens and potential N emissions, estimated both from annual and heterogeneous agricultural areas (Fig. 3) and from cattle census data (Fig. 4). Annual and heterogeneous agricultural types are the most intensive agricultural types, and include farming with fertilizer use, and also complex agricultural areas with cattle barns interspersed with greenhouses. All these agricultural activities, including cattle husbandry and farming, are known sources of NH_3-N (EPER, 2004; Galloway et al., 2003). Once emitted from agricultural fields or barns, N can be taken up by vegetation. It has been shown that lichens (Branquinho et al., in press; Frati et al., 2007) and bryophytes (Pitcairn et al., 2003) located near cattle barns, and plants located in forests near fertilized agricultural fields (Pocewicz et al., 2007), have higher N concentration in the thalli. N accumulation in lichens has therefore been used to monitor N deposition in local studies (Branquinho et al., in press; Frati et al., 2007; Olsen et al., 2010). However, those monitoring surveys were performed at a local level, not at a regional one, such as here.

Can N concentrations in lichens be used to estimate N emissions at high spatial resolution? N concentrations in lichens were significantly related to the main sources of atmospheric reduced N (Fig. 3, 4). This correlation was found at a regional scale, using two separate ways of calculating reduced N-emissions: area occupied by agriculture and NH_3 emitted by cattle. Additionally, the plotting of the significant correlations between N concentration in lichens and agricultural land-cover areas (Fig. 3) identified the areas under the influence of each agricultural type within the given neighbourhood. Therefore, mapping of N concentration in lichens may help locate areas with the highest N atmospheric deposition, identifying areas for monitoring potential exceedance of Critical Loads and Biodiversity impacts. In fact, we could make use of the interpolated N concentration in the studied region (Fig. 2), focusing on an important area for nature conservation (Fig. 5). We could observe that N concentration presented a patchy distribution, highlighting the short-range nature of N pollution. The Natura 2000 site "Comporta/Galé" (Fig. 5) is characterized by costal dunes habitats and costal lagoons, which are classified as Special Protection Areas. Up-stream of some of those lagoons we can find rice cultures, known to be important sources of NH_3 (Yan et al., 2003). Those rice fields are the probable source of increased N concentration observed in lichens near the Special Protection Area of "Lagoa de Santo André" (Fig. 5). This area is likely to be under strong N-pollution and should be particularly monitored for its impacts. Therefore mapping N concentrations in lichens provided an efficient way to determine the critical areas probably affected by N pollution and should be considered critical areas for monitoring biodiversity. In this way we reduce cost focusing the plant and animal diversity monitoring mainly the areas with potential high atmospheric N deposition.

CONCLUSION

Nitrogen concentrations measured in lichens were shown to be significantly correlated with the main regional sources of reduced N. They could therefore be used as an ecological tool to map, with high spatial resolution, the areas experiencing higher deposition of N and evaluate impacts on ecosystem functioning and biodiversity.

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chapter 8

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08 | Causes of change in nitrophytic and oligotrophic lichen species in a Mediterranean climate: impact of land-cover and atmospheric pollutants

ABSTRACT

With the aim of determining the main drivers of changes in nitrophytic and oligotrophic macro-lichen communities in an industrial region with a Mediterranean climate, we considered both land-cover types and atmospheric pollutants. We determined the relation between the abundance of nitrophytic and oligotrophic species with environmental factors considering the distance of influence of land-cover types. The results showed that oligotrophic species decreased in the proximity of artificial areas, barren land and agricultural areas, associated with higher concentrations of NO_2 and Zn, and Ti, probably dust of industrial and agricultural origin. Nitrophytic species were positively related to all the mentioned land-cover types, and with higher concentrations of Fe and N. Magnesium, probably from ocean aerosols, was negatively related to oligotrophic species and positively to nitrophytic.

INTRODUCTION

Mediterranean landscapes are characterized by a mosaic of different land-cover types, most of which are man-made. Pollutants may be emitted from some of these land-cover types, most notably urban and industrial but also agricultural. In Mediterranean areas, we must also considered that barren land (areas without vegetation) can also be responsible for the emission of some pollutants, mainly soil particles, dispersed as dust during the dry season. Although some studies have focussed on the impact of pollutants on natural and semi-natural ecosystems (Branquinho et al., 2008), few have tried to establish a linkage between land-cover types, the associated emission of pollutants and their effects on ecosystems (Frati et al., 2006; Gombert et al., 2004; Jovan and McCune, 2005; Purvis et al., 2003).

Lichens, organisms which result from a symbiosis (Honegger, 1991), are among the more sensitive to environmental changes, and it is not surprising that alterations in their communities are one of the most commonly tools used for ecosystems assessment and biomonitoring. Numerous biomonitoring studies using lichen diversity have been carried out worldwide, confirming that sensitive species decline in polluted areas, and tolerant species remain (Geebelen and Hoffmann, 2001; Geiser and Neitlich, 2007; Giordani et al., 2002; Nimis et al., 1991; Pirintsos and Loppi, 2003; Vokou et al., 1999). Monitoring approaches include measuring variations in lichens' diversity and/or abundance, physiological parameters, and/or their accumulation of pollutants (Branquinho, 2001).

An approach based on functional groups (that is, species that share some functional characteristics and thus react similarly to environmental factors) seems to be the most adequate for measuring environmental changes using biomonitors, because it is a balance between the very robust indicators that combine all species (such as total number of species) and a more detailed analysis, given by individual species. For example, lichen groups such as the "nitrophytic" and "acidophytic" have been studied and compared under NH_3 or agricultural pollution (Frati et al., 2007; Jovan and McCune, 2005, 2006; Ruisi et al., 2005; Ruoss, 1999; van Herk, 2001; Wolseley et al., 2006). Therefore an approach based on functional groups should provided a robust but still detailed response (Boutin and Jobin, 1998; McIntyre et al., 2003; van Diggelen et al., 2005).

It is well known that environmental factors affect living organisms at different spatial scales (Maurer, 2002). As a consequence, scale influences our perception of any phenomena under study (Dungan

et al., 2002; Maurer, 2002). During the development of this work it was necessary to include an analysis at different scales in order to better characterize the relations between lichen diversity and environmental factors. The term "scale" was used in its ecological sense: the level at which phenomena are measured, perceived or represented (Morinson and Hall).

Detailed analysis of spatial data requires the use of geostatistical methods, which have been shown to be important tools for vegetation ecology and biomonitoring studies. A geostatistical study of the spatial characteristics of biomonitoring variables can provide insights through the interpretation of variograms (Mitchell et al., 2000). Importantly, geostatistics also allows development of spatial models of variables, which can be used in interpolation with known uncertainty (Soares, 2000; Store and Jokimaki, 2003), provide generalization and scaling up methods (Burrough, 2001).

In general, the changes induced by human activities in ecosystems can more obviously be attributed to the concentrations of pollutants. However an approach that takes into consideration not only the concentration of a particular pollutant but also its source should aid impact studies and planning. Several authors have related lichen variables with neighbourhood land-cover (Aarrestad and Aamlid, 1999). Others authors (Tommervik et al., 1998), considered land-cover types as potential sources of pollutants, taking into account the distance from these to lichen sampling sites. However they only considered short ranges, up to about 200 m.

The more important and studied types of neighbourhood land-cover that may induce changes in lichen-diversity are urban and industrial. Some authors related dioxins and furans measured in lichens to the land-cover up to 2 km away (Augusto et al., 2004) while others considered distance to roads and traffic intensity (Gombert et al., 2003), as well as associated NO_2 and NH_3 (Frati et al., 2006).

As concentrations of SO_2 decrease in urban areas, nitrogen is playing an increasing role in changing lichen communities (Purvis et al., 2003) (Hultengren et al., 2004). Agricultural activities have also been recognized as important sources of pollution. Distance to farms has been considered some authors (van Herk, 1999), and cattle grazing has also been recognized as a significant cause of change in lichen communities (Ruoss, 1999; van Herk, 2001; Wolseley et al., 2006). Land-cover types capable of emitting dust, more important in Mediterranean areas, must also be considered (Branquinho et al., 2008; Loppi and Pirintsos, 2000).

The objective of this work was to determine the main causes of changes in nitrophytic and oligotrophic macro-lichen communities in a region with high industrial activity and a Mediterranean climate.

MATERIALS AND METHODS

Study area

The study area (about 1500 km^2, 50x30 km) is located on the SW coast of continental Portugal, facing the Atlantic Ocean to the West. It includes two small mountains (Serra de Grândola, 383 m and Serra do Cercal, 378 m) that are located parallel to the coast, in north-south orientation. The annual average temperature is between 16 and 17.5ºC, the average annual precipitation is between 600 and 1000 mm and average annual insolation is 3000 h, averages from years 1931 to 1960 (IA, 2006)). The prevailing winds come from the N-NW. The study area lies in four municipalities: Grândola (population 14,901), Santiago do Cacém (population 31,105), Odemira (26,106) and Sines (population 13,577) (INE, 2001).

This area covers several important industrial facilities established since the late 1970's: a coal-fired power station, an oil refinery, a chemical plant and, more recently, an industrial landfill as well as many other smaller industries. The sea harbour allows the arrival of raw materials such as coal, oil, gas and other chemicals. There are two main motorways and one railway. Urban development of the seaside towns has recently increased. Most of the inland landscape is dominated by agro-forestry

activities, mainly cork-oak woodlands (*Quercus suber* L.). A natural reserve area, Parque Natural do Sudoeste Alentejano e Costa Vicentina (PNSACV) is located in the SW.

Biodiversity data collection and calculation of lichen diversity indicators

Lichen diversity data was collected as described elsewhere (Pinho et al., 2004). Briefly, lichen vegetation growing on the trunks of *Quercus suber* L. trees was sampled at 71 sites for foliose and fruticose species according to a standard methodology (Asta et al., 2002). This method uses a grid with five 10x10 cm squares, placed on the four main orientations of the trunk. All foliose and fruticose species growing inside the squares were sampled, and the number of squares on which they occurred was recorded as their frequency. Two lichen-diversity indicators based on functional groups were calculated for each sampling site: LDV (lichen diversity value) of nitrophytic species "LDVnitro" and of oligotrophic species "LDVoligo". Lichen diversity values (LDV) were calculated (Asta et al., 2002), only using the species belonging to the specific functional groups. Division of species into the two functional groups was carried out according to the eutrophication maximum classification of (Nimis and Martellos, 2008). Species with an eutrophication index of 1 or 2 were considered oligotrophic, species with an eutrophication index of 4 or 5 were considered nitrophytic. LDVmeso included species classified with an index of 3. Note that the use of macro-lichens to calculate LDV values could result in some noise in the data, although macrolichens have been shown to present similar trends to crustose lichens, at least in terms of land-use intensity (Bergamini et al., 2005). LDVoligo data followed a near-normal distribution at the regional scale of analysis, no data was excluded and no transformation was required. LDVnitro data also followed a near normal distribution at least at the local level of analysis in the areas with significant correlations, from which conclusions were taken.

All data was collected as a part of the SinesBioar project (LIFE00 ENV/P/000830, project site www.ccdr-a.gov.pt/sinesbioar) which was intended to characterize air quality in the region. Results of air quality analysis can be found elsewhere (Pinho et al., 2004).

Pollutant concentrations

Lichens are sensitive to many environmental factors, some of which are site-variables (such as tree species) while others are regional or local-variables, according to their area of influence within the study area. The importance of site-variables was previously tested and it was found that differences between sites were not due to site-variables but rather to local or regional environmental factors (Pinho et al., 2004). We considered the influence of land-cover and pollutant concentrations as local or regional environmental factors.

We determined the concentrations of several pollutants at each sampling site using two methods: diffusion tubes and biomonitors. Diffusion tubes were placed in the study area for 15 days, and analyzed for NO_2 concentration. In order to understand how the results of the 15 days' sampling could be related to the annual emissions of NO_2, we compared them to the climatic and NO_2 data from permanent local air monitoring stations. It was found that both the emissions and climatic data of this 15 day period were similar to the prevailing conditions, therefore we could assume that the resulting concentrations and dispersion observed by the diffusion tubes were representative of the year emissions. For biomonitoring the concentrations of Si, Ni, Mn, Ca, Cr, Al, K, Fe, Co, Ti, Zn and Mg were measured in the lichen *Parmotrema hypoleucinum* (J.Steiner) Hale, collected from cork-oak trees at each sampling site. After collection, the lichens were stored in plastic bags and transported to the laboratory, where the unwashed samples were immediately dried at room temperature and sorted to remove extraneous material. Special care was taken when sorting in order to select only the chosen species, to avoid errors due to the presence of other species. The cleaned samples were then ground (Glen Creston Ltd. MM 2000) and separated into two parts: one for metal analysis and another

for S and N. For S and N analysis, ground lichen samples were dried at 50ºC for 24h. Three replicates of each sample were separated (2.5 mg for S analysis and 1 mg for N analysis) in a high performance balance (Sartorius Microanalytical Balance) and analyzed by elemental mass analysis (Euro Vector CHNS-O Elemental Analyzer). The standards used were atropine ($C_{17}H_{23}NO_3$ with 4.84% N) for nitrogen analysis and BBOT ($C_{26}H_{26}N_2O_2S$ with 7.44% S) for sulfur analysis. The precision of analysis was 0.03% for S and 0.07% for N, and the accuracy was 0.02% for S and 0.01% for N. For metal analysis, ground and dried samples were quantified by ICP-OES. The precision and accuracy of the analysis was checked against reference material. These metal analyses took place in the British analytical laboratory Tes Breatby.

Land-cover map

A land-cover classification was made for the entire region (50 x 30 km) by assisted classification of LandSat images (30 m resolution, multispectral, acquired in spring of 2001). The land-cover types were classified in terms of their potential for emitting nitrogen compounds: 1) "Annual Cultures", corresponding to areas with annual agricultural activities, with potential emissions of N mainly in the form of NH_3 and of dust; 2) "Barren-lands", corresponding to areas with little or no vegetation, with potential emissions of soil dust, especially in summer, for example quarries; 3) "Artificial", corresponding to areas with urban, industrial and road uses, which may be associated with the emission of many industrial and urban pollutants, including NO_x. Other occurring land-cover types (water, olive cultures, herbs and shrubs, pine forests and broad-leaf forests) although classified, were not considered for this analysis. Land-cover variables were calculated as the absolute area (m^2) occupied by each land-cover type contained by a circle of varied radius centred at each sampling site. With this classification it was possible to characterize the land-cover in the region and therefore determine the possible influence of neighbourhood land-cover on lichen-diversity indicators as explained below.

Geostatistical analysis of lichen-diversity and pollutants data

After a variogram analysis and manual fitting of interpolation functions, the lichen diversity data and pollutant concentrations were interpolated using ordinary kriging (Soares, 2000). This interpolation attempted to map each indicator and perform a geostatistical analysis, and was necessary because pollutant and lichen diversity sampling sites did not always match. Thus, for the following analysis we considered estimated values of pollutant concentrations at lichen diversity sampling sites.

Analysis at a regional scale

Regional scale analysis was performed by evaluating the linear and non-linear correlations between variables across the region: LDVoligo vs. land-cover class; LDVnitro vs. land-cover class; LDVoligo vs. pollutant concentration estimates; LDVnitro vs. pollutant concentration estimates. To evaluate linear correlations, the Pearson's coefficient was calculated, while to evaluate non-linear relations, exponential functions were fitted to data. The square residuals (R^2) were used as a measure of the relations' strengths. The land-cover variables were defined as the area occupied by a certain land-cover type existing in the neighbourhood of each lichen-diversity sampling site, and pollutant variables as the pollutant concentrations estimated for each lichen diversity site. The extent of the influence of neighbourhood land-cover types was ascertained by determining the area occupied by each land-cover type in a series of circles around each lichen-diversity sampling site, of increasing radius between 150 m and 6 km. For each circle size the correlation between lichen-diversity indicators and land-cover types was evaluated. The distance for which the greatest correlation (positive or negative) was found was then considered to be the neighbourhood size which maximized the correlation between variables. We assumed that if the correlation increased in significance with increased neighbourhood size, then those additional areas were still affecting

lichen-diversity. If the correlation became lower it was considered that they were not (Pinho et al., 2008).

Local scale analysis

The results from the regional scale analysis described above as well as other analyses of lichen diversity at the same site (Pinho et al., 2004) suggested that the relation between LDVnitro and land-cover types could be better addressed at a local scale rather than at a regional scale. Instead of evaluating the global correlation, the analysis at a local scale was made by calculating local correlation coefficients (Pearson's correlation coefficient) using moving windows over the entire region, obtaining a measure of correlation for each sampling site. In this case a circular window with 10 km radius around each sampling site was used, and the results were tested for statistical significance and mapped for the study area. A 10 km buffer was defined around each sampling site and all sampling sites contained within the buffer were used to calculate the correlation coefficient value. Correlation coefficients values (one for each sampling site) were then tested for statistical significance (considered significant for $p<0.05$) and mapped for the study area using discrete symbols. A 10 km radius was chosen in order to obtain a new but significant scale of analysis. Larger radii were tested but included most of the study area (and most of the sampling sites), so correlation coefficients tended to be similar to the regional ones. Smaller radii included too few sampling sites, and most correlation coefficients were considered not significant due to the low number of samples used. Note that each correlation between variables corresponded to one map and that the number of samples used to calculate correlations was different for each sampling site (Pinho et al., 2008). Note that when determining the influence of neighbourhood land-cover, there is a neighbourhood size for which the correlation coefficient is a maximum, as in the regional scale analysis. Therefore, as in the regional scale analysis, a series of neighbourhood sizes were tested, from 150 m to 6600 m radius around sampling sites, and that which maximized the correlation coefficient was determined and plotted on the map. Only sites for which a significant correlation was found were plotted, irrespective of the neighbourhood size used.

Multivariate analysis

In order to simultaneously analyse lichen-diversity indicators, pollutants and land-cover types we performed a Redundancy Analysis (RDA) using LDVnitro, LDVoligo and LDVmeso as variables to be explained. Several analyses were made, firstly using pollutants, then land-cover, with the intention of giving preference to pollutants as explaining variables, since they have a direct impact on lichen-diversity indicators. Initially, all pollutants were submitted to the RDA, using stepwise choice of the explaining variables, then land-cover variables (all distances) were also submitted, under a stepwise procedure. The final RDA consisted of the choice of the 4 pollutants and 1 land-cover variable. All other pollutants and land-cover types were plotted as passive variables.

Software used

Geostatistical analysis of lichen-diversity variables (variography) was carried out using GeoMS (CERENA, 2000) and interpolated by ordinary Kriging using the same software. Correlations were determined with Statistica 7 (StatSoft, 2004). A software routine in Fortran was developed by the authors for calculating local linear correlation coefficients based on a moving windows algorithm. All GIS and mapping operations and output maps were prepared with ArcMap 9.1 (ESRI, 2001). Databases were managed using Excel 2003 and Access 2003 (Microsoft, 2003).

RESULTS

Spatial structure of the data and mapping of lichen-diversity indicators

In order to analyse their spatial structure, the variograms for both lichen-diversity variables were calculated (Figure 1) and then fitted to a function summarising the spatial characteristics of each variable (Table 1). These functions were used to interpolate and map both variables for the study area (Figure 2).

The environmental factors underlying the two biodiversity variables showed different spatial characteristics. The abundance of nitrophytic species (LDVnitro) showed smaller spatial continuity (A_1+A_2 in Table 1), 8000 m than LDVoligo (80000 m). Moreover, the abundance of oligotrophic species (LDVoligo) required two structures to correctly model its spatial characteristics (designated 1 and 2 in Table 1), as opposed to a single structure for LDVnitro. This indicates the potential presence of two different groups of environmental factors mainly affecting the LDVoligo variable.

The variable LDVnitro showed lower anisotropy (1.778) in the North-East - South-West direction, whereas LDVoligo showed a stronger anisotropy (6.667 in the second structure) in the North-South direction (Table 1), the latter being in agreement with the prevailing winds.

Despite the differences found previously between the variables, similar ranges were found for the first structure of the variogram, which could be related to the fact that same environmental factors might be influencing both variables (Table 1) at shorter distances.

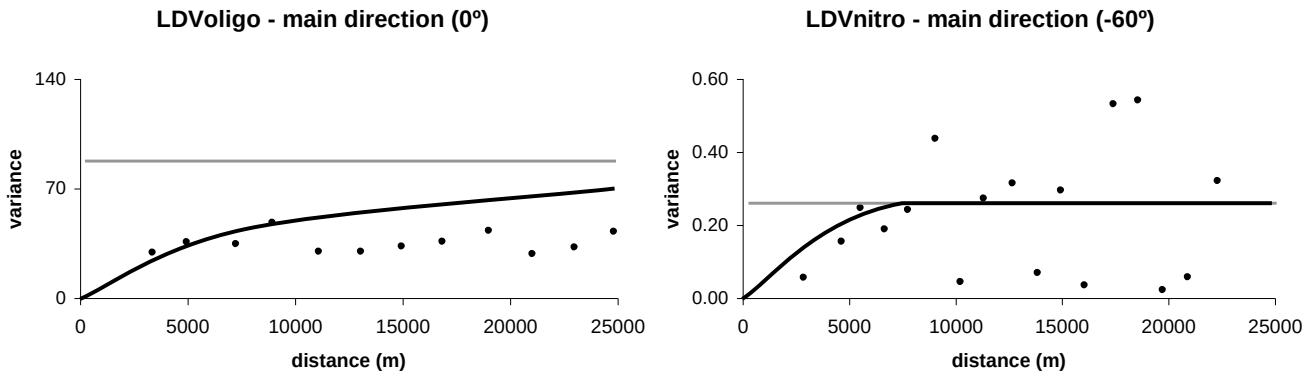

Figure 1: Variograms and functions used in the interpolation of the two lichen-diversity indicators (LDVoligo and LDVnitro). Function characteristics are indicated in Table 1.

Table 1: Spatial characteristics of the fitted function to each of the lichen-diversity variables. C_0: nugget effect; $C_{1,2}$: partial sill (m); $A_{1,2}$: range (m); $anisotropy_{1,2}$: main direction/minor direction; total C: total variance. All functions were spherical.

lichen-diversity variable	C_0	C_1	A_1	$anisotropy_1$	C_2	A_2	$anisotropy_2$	total C	main direction
LDVnitro	0	0.260	8000	1.778				0.260	-60º (W-NW)
LDVoligo	0	46.8	11000	1.294	40.68	80000	6.667	87.49	0º (N)

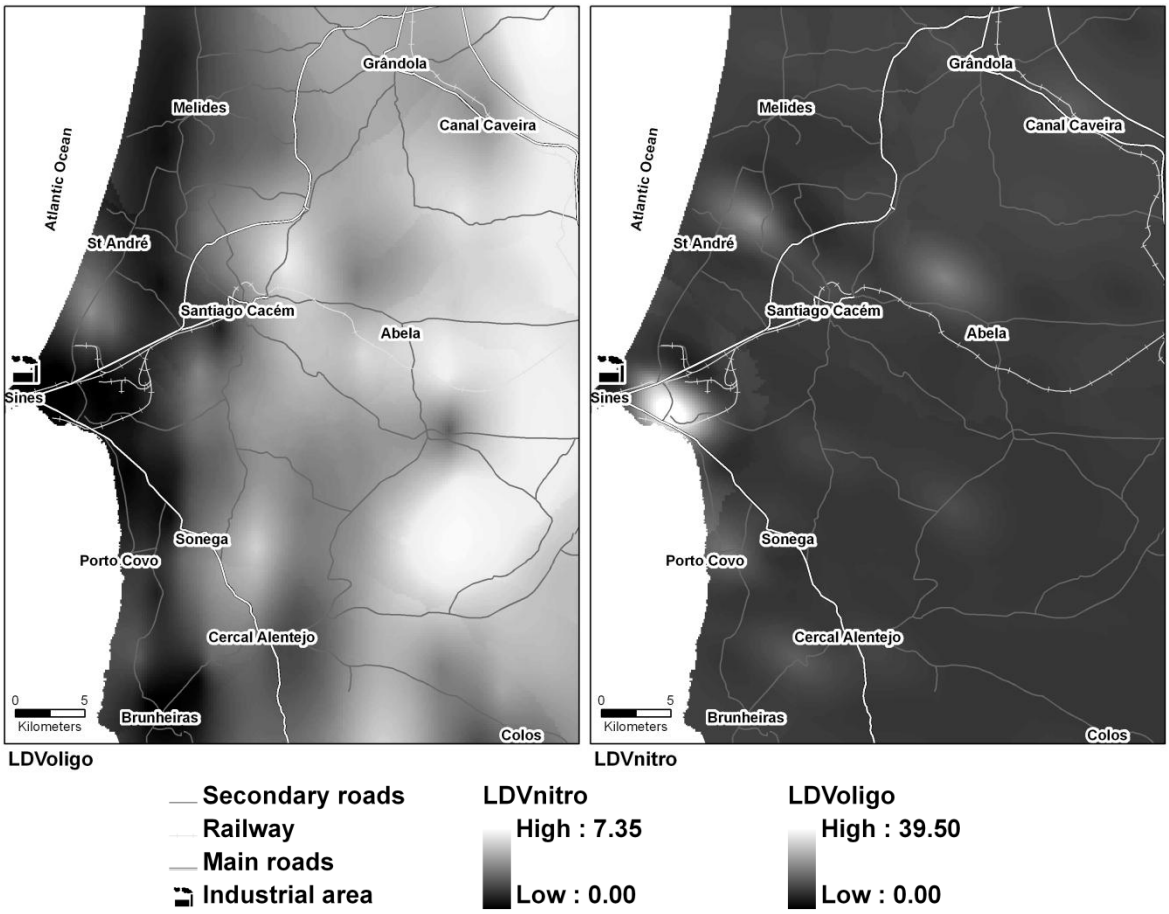

Figure 2: Map of the interpolation of two lichen-diversity indicators, LDV of oligotrophic species and LDV of nitrophytic species. Lighter colours represent higher abundances of the indicators, darker colours represent lower abundances.

The function depicted in Table 1 was used to interpolate lichen-diversity variables, obtaining the map for the entire study area (Figure 2). In general, LDVoligo values were greater inland and lower near the coast. This lichen-diversity indicator presented the lowest values near the industrial area, reaching zero. On the other hand LDVnitro showed the highest values at the sampling sites near the industrial areas, and at some other sites scattered across the study area, however it also reached zero in the industrial area.

In order to verify whether the spatial distributions of LDVnitro and LDVoligo were related, the conditional distribution of LDVnitro given LDVoligo was calculated (Fig. 3), by dividing LDVoligo into classes. Generally, the median and interquartile ranges of the conditional distribution of LDVnitro decreased as LDVoligo levels increased, i.e., the levels of LDVnitro tended to be lower and less spread around the median as the levels of LDVoligo increased.

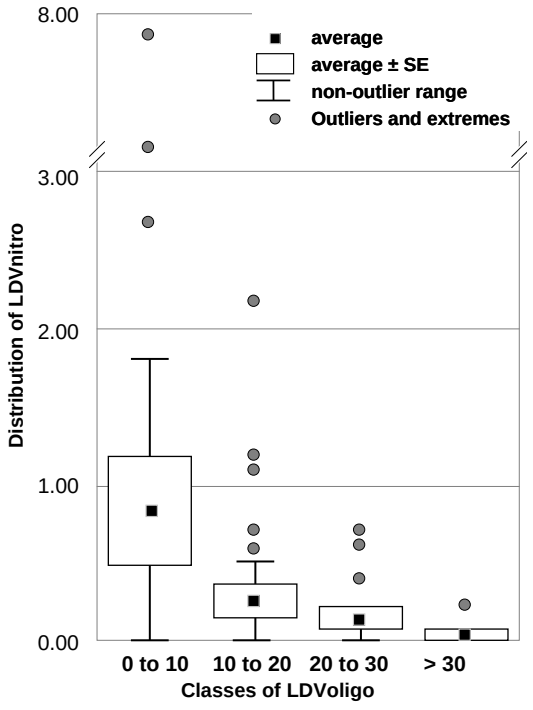

Figure 3: Distribution of the values of nitrophytic species (LDVnitro) in classes of oligotrophic species (LDVoligo), using box-plots representation. SE: standard error.

Multivariate analysis of pollutants and land-cover

In order to understand how the environmental variables contributed simultaneously to the distribution of the lichen-diversity indicators, two sequential redundancy analyses (RDA) were performed. The first considered only the pollutants, whereas the second used the pollutants selected in the first analysis together with the land-cover variables. The first analysis (data not shown) explained 39.7% of the lichen-diversity indicators' variance, and selected the concentration of Ti, NO_2, Mg and Zn as the main causes for changes in lichen species (data not shown). For the second RDA, the variable Annual Cultures (considering a neighbourhood with a radius of 1800 m) was added to explain the lichen data. This final RDA explained 56.6% of lichen-diversity indicators' variance. We found that higher concentrations of Ti, Zn, Fe, Co, NO_2, S and Mg were positively related to Artificial, Barren and Annual Cultures land-covers (Figure 4).

LDVoligo showed the highest variation within the studied environmental gradient, and was negatively influenced by most of the indicator elements and pollutants, especially Ti, NO_2, Mg and Zn. In addition to those pollutants, the variable Annual Cultures was also negatively correlated to LDVoligo. LDVnitro showed a much weaker relation to this group of variables, although it could be positively related to most of them. LDVmeso was mainly related to the second axis, which represented only 4.2% of the extracted variance and could not be associated with any of the studied variables.

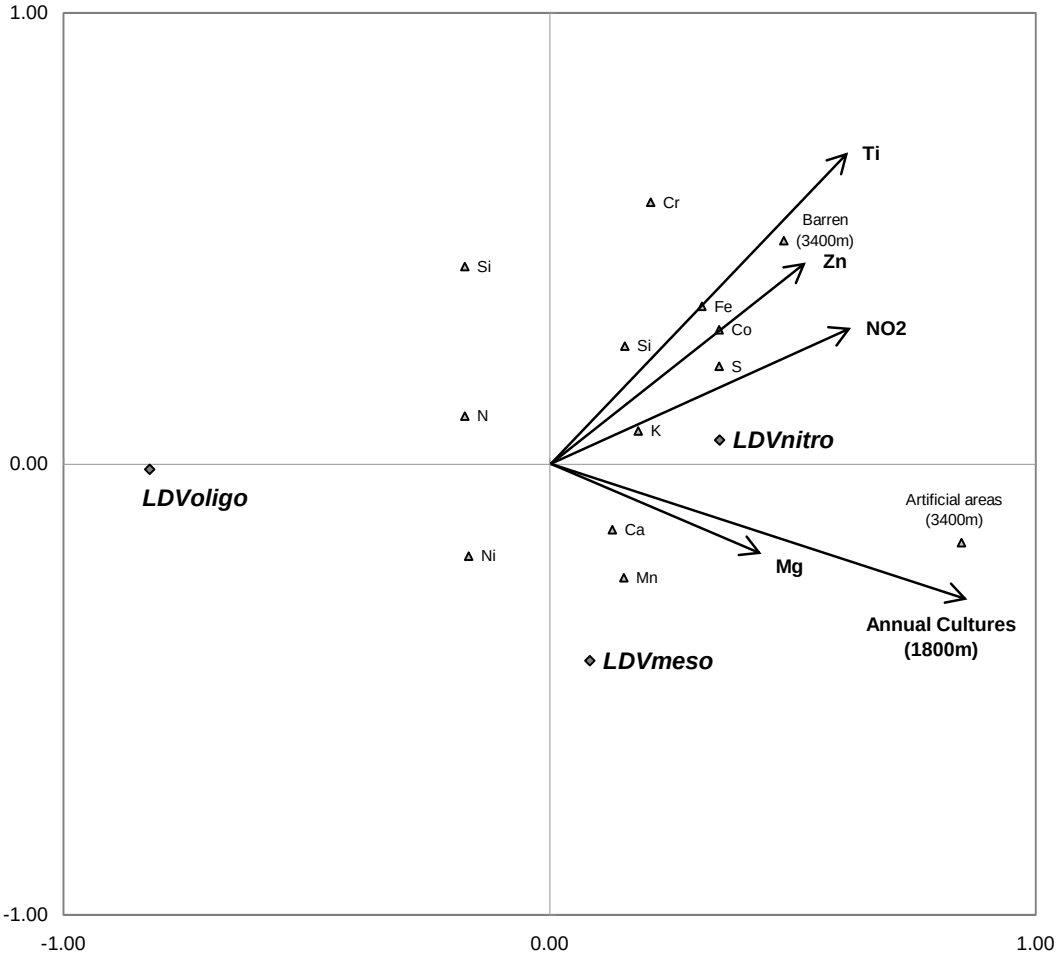

Figure 4: RDA of lichen-diversity indicators, pollutants and land-cover. The first axis represents 52.4% of variance, and the second 4.2%. The RDA resulted from a sequential analysis introducing first the pollutants and then the land-cover types, under a stepwise procedure. The environmental variables not included in the final model were projected as passive variables.

Influence of land-cover in lichen-diversity indicators

Neighbourhood land-cover was expected to be correlated in some way with lichen-diversity indicators. At a regional scale of analysis, we plotted the relation between LDVoligo and the area occupied by each land-cover type in the neighbourhood of sampling sites (Fig. 5A). Radii between 150 and 6600 m were considered and the one that resulted in the highest correlation, using an exponential function, was chosen and plotted. The radius that best fit were 600 m for Annual Cultures, 1000 m for Artificial and 1800 m for Barren. All land-cover types resulted in highly significant negative relations (R^2 above 0.51), indicating that the presence of any of the tested land-cover types could influence LDVoligo.

The best relations between concentrations of elements and pollutants versus LDVoligo are plotted in Figure 5B: Ti, NO_2, Mg and Zn. The highest concentrations of these elements were related with lowest levels of LDVoligo.

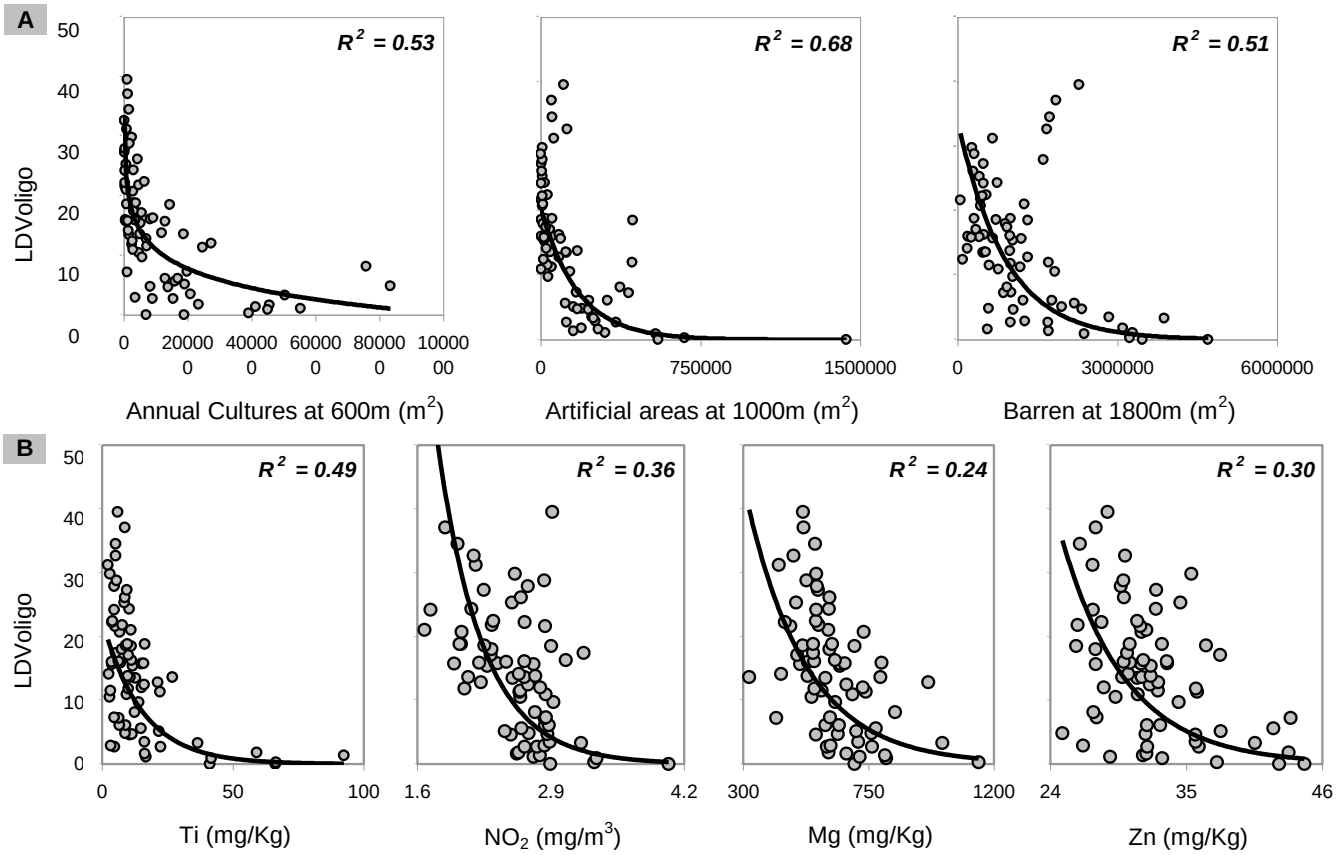

Figure 5: Plot of the relation between lichen-diversity indicators and: land-cover types (A) and pollutants and elements (B). The fit lines are exponential functions and R^2 (the square residuals) were used as a measure of the relations' strengths. The distances chosen for land-cover types were those which resulted in the highest correlation coefficients. Ti, Mg and Zn were measured in lichens and NO_2 using diffusion tubes.

The same analysis performed with LDVnitro resulted in non-significant or very weak correlations (R^2<0.20, data not shown). Since the variogram (Fig. 1) and map (Fig. 2) of this variable suggested that changes were occurring at a smaller spatial scale, we decided to analyse the relation between land-cover, pollutants and LDVnitro at a local scale (Fig 6). The local correlation analysis for LDVnitro resulted in much higher correlation coefficients than at a regional scale; many sampling sites showed correlations over 0.60. The correlations were always positive, showing that Barren, Annual Cultures and Artificial land-covers in the neighbourhoods of sampling sites could be related to increasing LDV of nitrophytic species. Annual Cultures land-cover was more significantly correlated to LDVnitro in the north of the study area. LDVnitro was also correlated to Artificial land-cover near the industrial

zone and to Barren land-cover along most of the coast, to the North and South (Fig. 6).

The relation between LDVnitro and pollutants was also calculated at the local scale (Fig. 6). Nitrogen, Mg and Fe concentrations in biomonitors showed significant positive correlations with LDVnitro in some areas (Fig. 6). No other pollutants showed significant correlations (data not shown), not even NO_2. This pollutant showed few areas with significant positive correlation with LDVnitro, but these occasional and dispersed points were difficult to interpret.

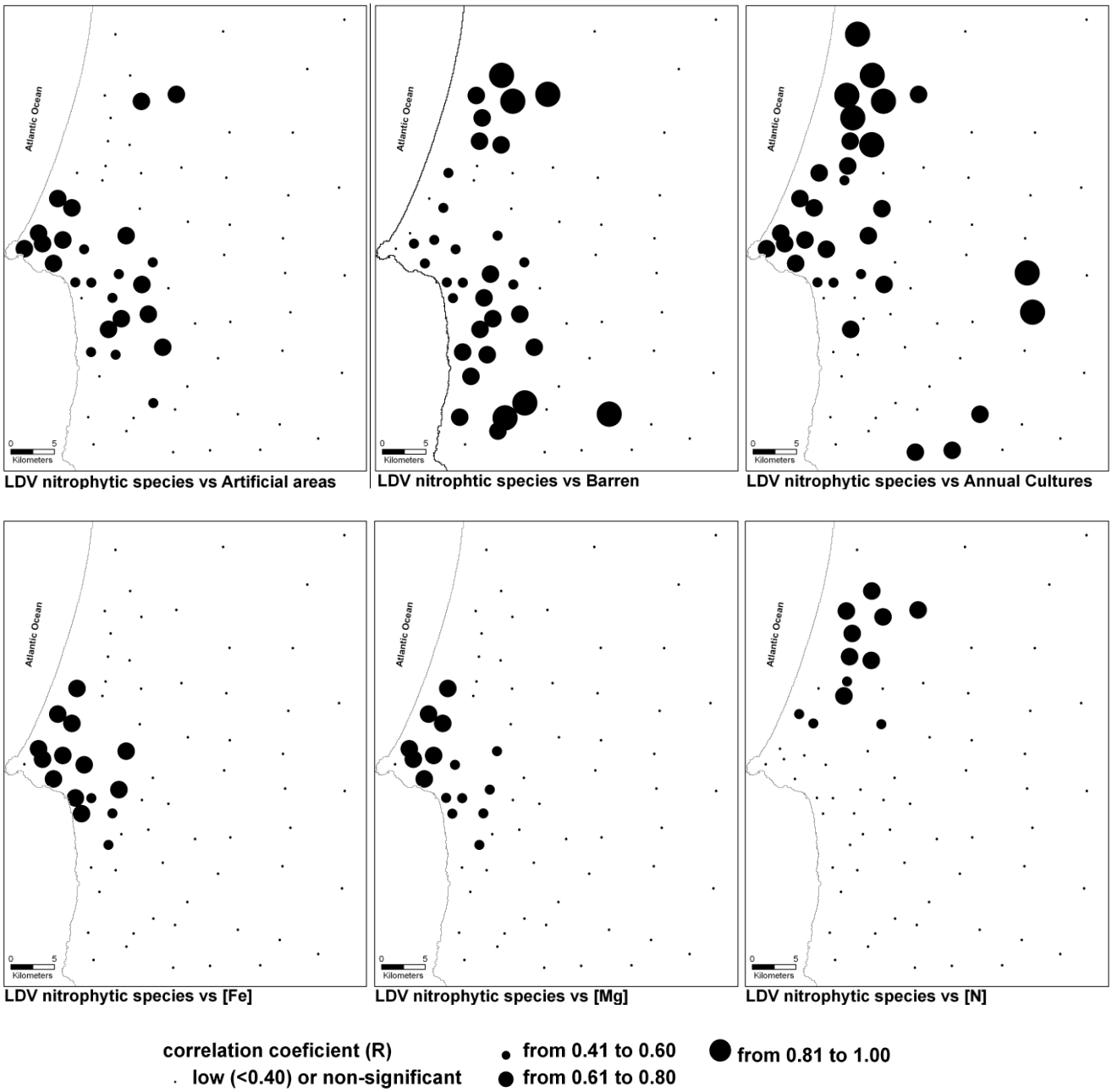

Figure 6: Mapping of local correlations between lichen-diversity indicators and land-cover types present in the neighbourhood of sampling sites (above) and pollutants (below). Correlations were calculated for each sampling site using all sites within 10 km, and all statistically significant correlations were plotted using larger symbols. For land-cover types, the plotted correlations were obtained using the distance for which the maximum correlation coefficient was obtained.

DISCUSSION

Effect of elements and land-cover

The classification of lichens (Nimis and Martellos, 2008) is based on lower (oligotrophic) and higher (nitrophytic) tolerance to nutrients, especially nitrogen. Since the lichen diversity indicator LDVoligo decreased (Fig. 5) and LDVnitro increased (Fig. 6) with increasing areas of Barren, Artificial and Annual Cultures land-covers in the neighbourhood, we suggest that some kind of stress produced by these land-cover types influences lichen communities. Oligotrophic lichen species decreased exponentially with increasing areas of Barren, Artificial and Annual Cultures land-covers in their neighbourhoods, and with increasing concentrations of Ti, NO_2, Mg and Zn (Fig. 5). This group of lichens responded similarly to the 3 types of land-cover studied (Fig. 5A). Perhaps the land-cover types are responsible for the emission of elements and pollutants which damage oligotrophic lichens, or that reduce their competitive abilities, for example their growth rates. In fact, we observed a significant exponential decrease with increasing concentrations of elements and pollutants, Ti concentration in lichen being that with the strongest correlation (Fig. 5B). Titanium and iron are indicators of atmospheric particulate material emitted from the soil by agricultural activities, from naked soil in windy areas, and industrial and vehicular emissions (Lopes et al., 2006). The impact of these dust particles on oligotrophic lichens could be either direct physical damage or indirect as a consequence of the chemical composition of the particles (Grantz et al., 2003). Most documented toxic effects of particles on vegetation are associated with their acidity, trace metal content, nutrient content, surfactant properties, or salinity (Grantz et al., 2003). Oligotrophic lichens were negatively related with Zn concentration in lichens (Fig. 5B). Zinc is usually associated with industrial pollution and vehicle emissions, which are high in the region due to the presence of intense heavy vehicles traffic, a refinery and a coal-fired power plant, among other industrial plants. Magnesium is a macro-element, not usually regarded as a pollutant, but can indicate sea-salt aerosol dispersion (Figueira et al., 1999). Interestingly, high concentrations of Mg in lichens were significantly correlated with low levels of oligotrophic lichens (Fig. 5B). Thus, Mg concentrations are reflecting the proximity to coastal urban areas, or to the ocean itself, which is a source of several other environmental factors of known impact on lichens, such as salinity and humidity.

On the other hand, nitrophytic lichens increased with increasing neighbourhood presence of Barren, Artificial and Annual Cultures land-covers, and with high concentrations of Fe and N (Fig. 6). Barren land-cover seemed to be that with the greatest impact on the nitrophytic lichens (Fig. 6A). This land-cover type includes open areas with naked soil that are important sources of dust particles, especially in the dry, windy summers. In fact we observed that Fe in lichens, an indicator of soil dust particles (Branquinho et al., 2008), was also significantly correlated with increasing nitrophytic lichens in the more industrial and urban areas (Fig. 6). This could be because dust is considered one of the main causes for the rise in bark pH of *Quercus* trees (Loppi and DeDominicis, 1996; Loppi et al., 1997), and as a consequence this could lead to a shift in the oligo/nitrophytic lichen composition (Frati et al., 2006). On the other hand, it seems that in the area with more agriculture, the atmospheric dust particles are enriched with nitrogen (Fig. 6), since the main source of atmospheric nitrogen in agricultural areas is NH_3 (Bleeker and Erisman, 1998). Several authors have found a good relation between N in lichens and NH_3 in the atmosphere (Frati et al., 2006; Gaio-Oliveira et al., 2001; Kauppi, 1980; Ruoss, 1999; Søchting, 1995), but poorer relations have been found with atmospheric NO_X (Vingiani et al., 2004). Our results (Fig. 5B) are in accordance with previous findings that atmospheric NH_3, shifts lichen communities from oligo- to nitrophytic functional types (Frati et al., 2006; Jovan and McCune, 2005; Ruisi et al., 2005; Ruoss, 1999; van Herk, 2001; Wolseley et al., 2006).

We have therefore found that lichen communities were affected by: i) industrial pollutants such as Zn, that cause a depletion in oligotrophic lichens but have no effect on nitrophytic; ii) dust particles, as

indicated by Ti and Fe, that cause a depletion in oligotrophic species and an increase in nitrophytic; iii) proximity to the coast, which has a negative impact on oligotrophic species but none on nitrophytic.

Concerning the impact of NO_2 on lichens, our study showed that this pollutant was one of the most important predictors for changes in oligotrophic lichen communities (Fig. 4), whereas S concentration in lichens was only moderately associated with those changes (it was not selected as an important variable in the RDA). Nitrophytic species did not show a significant correlation with NO_2 (Fig.6), but were however able to survive under high concentrations. This group of lichens was often found at locations where the highest concentrations in the diffusion tubes were detected, namely industrial and urban areas (Fig. 4 and 6B). The annual average concentrations of NO_2 (2004, continuous monitoring) were 7.4 $\mu g\ m^{-3}$ in industrial areas ("Sonega") and 2.9 $\mu g\ m^{-3}$ in rural areas ("Monte Velho").

As for sulphur, several works have reported good correlations between atmospheric SO_2 deposition and total S concentration in lichens (Nash and Gries, 2002). However, lichen diversity was not significantly correlated with S content in lichens, in spite of the proximity of a significant source of SO_2, the coal-fired power plant. The 2004 annual averages of SO_2 ranged between 13.4 $\mu g\ m^{-3}$ in an industrial area to 9.0 $\mu g\ m^{-3}$ in a rural area (IA, 2006). The reduced impact of SO_2 on lichen communities could be due to the wide dispersion of this pollutant and consequently its dilution, since the power plant chimneys are among the highest in Europe, at 225 m above ground level.

The impact of NO_2 on lichen communities is not clear, because most studies of this matter also observed an association with SO_2 (van Dobben et al., 2001), making it particularly difficult to isolate the effect of NO_2. These authors found that under SO_2 and NO_2 pollution, all species (except *Lecanora conizaeoides*) decreased significantly. Some other studies that tried to isolate the impact of NO_2 did not find significant relations between NO_2 concentration and lichen diversity, even with NO_2 concentrations similar to those observed in the present study (Frati et al., 2006; Gombert et al., 2004). Since we did not observe a relation between lichens and SO_2 but did find a relation between lichens and NO_2, we can consider that in our study area SO_2 pollution was not an important stress factor. Specifically, the effect of NO_2 on lichen communities was a decrease in abundance of oligotrophic species (Fig. 5) and no clear effect on nitrophytic. Additionally, NO_2 was not found to be a source of N for these species (Fig. 6).

Changes in the distribution of oligotrophic vs nitrophytic lichens

In general, we found that under less-disturbed environmental conditions, and for maximum LDV of oligotrophic lichens, the LDV of nitrophytic species is at a minimum, as many authors have already found (Wolseley et al., 2006). However, under more disturbed conditions, which correspond to lower LDVoligo, the pattern is much more variable (Fig. 3): the LDVnitro ranges from the minimum (0) to the maximum (7.4) values found in this study (Fig 2). This shows that the simple absence of oligotrophic lichens is not enough to ensure more abundance of nitrophytic species. Thus we suggest that under disturbed conditions there are factors which are simultaneously adverse for oligotrophic and promote nitrophytic species; while other environmental factors are adverse for oligotrophic but have no effect on nitrophytic species.

The high spatial continuity and anisotropy in the direction of the prevailing winds of the lichen-diversity indicator LDVoligo (Fig. 1 and Table 1) showed that the environmental variables influencing the distribution of this indicator are present in most of the study area, and that their distribution is mainly governed by the prevailing winds. Since oligotrophic species are very sensitive to increasing levels of gaseous pollutants such as NO_2 and SO_2 and to aerosols from sea, the smaller size of these particles may allow the dispersion of the pollutants over large distances in the prevailing wind direction. In contrast, LDVnitro displayed a much shorter

spatial continuity, and a weak anisotropy that was not in the direction of the prevailing winds (Fig. 1 and Table 1). Moreover we could only find significant correlations with environmental factors at a local scale (Fig. 6). These differences in the range of influence on nitrophytic and oligotrophic species might be related to the fact that environmental variables influencing the distribution of this indicator were only present in some sections of the study area. On the other hand, nitrophytic species might require a rather large increase in pollutant concentrations before an effect can be observed. High concentrations of these pollutants must be restricted to small areas. Another possibility is that particles size that influence nitrophytic lichen species are on average larger than those which influence oligotrophic. The former might be supported by the data in Fig. 6, which shows that nitrophytic species were promoted in areas with increased Barren land-cover or dust deposition (Fe concentration in *in-situ* lichens), which is associated with large particles (McTainsh et al., 1997; Morawska et al., 1999). The same occurs with particulate material, most probably dust, which is produced in agriculture areas. This land use is related with an increase in total nitrogen in lichens and also promotes nitrophytic lichen species. In fact, nitrophytic lichens do not have any significant relation with gaseous pollutants such as NO_2 (Fig. 6B), highlighting the importance of the pollutant particle-size for these two different functional groups.

CONCLUSIONS

In this work, developed in a region with multiple disturbance sources and large industrial facilities, we have determined that barren, artificial and agricultural areas associated with higher concentrations of Ti, NO_2, Mg, Zn, Fe and N, were the main drivers for changes in nitrophytic and oligotrophic macro-lichen communities under Mediterranean climate.

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chapter 9: General Discussion

09 | General Discussion

Global change has multiple direct and indirect drivers (Rockstrom et al., 2009; Steffen et al., 2004) and causes multiple effects on planetary biomes (IGBP, 2007; Steffen et al., 2004). Evaluating the local effects of environmental changes due to natural and/or anthropogenic activities has been one of the most relevant research areas in the last decades. Moreover, under scenarios of global change it is crucial to provide a detailed evaluation of environmental changes at different scales (temporal and spatial) and under different levels of complexity (simultaneous interactions between several factors).

However, it is also clear that it is not possible to have a large number of physical-chemical monitoring stations, monitoring all possible environmental changes, especially when the time of change or even the presence of some of environmental factor is not known. Moreover, when monitored by physical-chemical stations, the results may still have lack of spatial resolution and there is no confirmation that the environmental change as caused a biological impact. Thus, the use of ecological indicators such as lichens can contribute to fulfil these information gaps, as well as, to identify critical areas, ameliorate the impacts or rank priorities in order to improve the efficiency of environment management actions.

ECOLOGICAL INDICATORS BASED ON FUNCTIONAL-DIVERSITY

Although frequently correlated with each other, the lichen-variables Number of Species (NrSp) and Lichen Diversity Value (LDV) (Asta et al., 2002) are rarely compared regarding their performance as ecological indicators (Perlmutter, 2010) and seldom studied when considering low-intensity environmental factors. In this work we showed that LDV, a measure that combines information of species richness and abundance, was more significantly related to changes in environmental conditions than only NrSp (Table 1). This could be related to the intensity of the environmental factors: low intensity versus high intensity. Thus a small environmental change could lead to a reduction in abundance whereas a stronger one could lead to species disappearance. This conclusion was based on the spatial analysis of both lichen-variables showing that the spatial range of LDV was generally much higher than the one of NrSp; for example more than 80 km for LDV and 9 km for NrSp (Table 2 in ch02.1). Accordingly, we observed a decrease in the LDV of oligotrophic species (LDVoligo) under increasing land-use intensity (Fig 5D in ch04), although the richness of these same species did not change (Fig 5C in ch04) While LDV and species richness are usually correlated (Nascimbene et al., 2007), LDV is not considered an adequate surrogate of species richness (Bergamini et al., 2005). The underlying reason for this could be that LDV responds to environmental factors of too low intensities to cause species loss. Thus, and accordingly to the results presented in this work we may say that the **lichen-variable LDV rather than species richness should be used as an ecological indicator of the impact of low-intensity environmental factors.**

Table 1: Comparison between the relations of environmental factors and two lichen-variables, one based on the number of species and the other also accounting for species abundance, Lichen Diversity Value (LDV). The number of samples was the same between each pair. LUI is the land-use intensity (see ch04). *** P<0.001, ** P<0.01, * P<0.05.

Variables	R^2
total number of species vs artificial areas (ch02.1)	0.23***
total LDV vs artificial areas (ch02.1)	0.37***
number of oligotrophic species vs LUI (ch04)	ns
LDVoligo vs LUI (ch04)	0.26***
number of nitrophytic species vs LUI (ch04)	0.36***
LDVnitro vs LUI (ch04)	0.40***

In accordance to what we found in this work other authors already reported that measures of total diversity such as total NrSp and total LDV can be influenced by multiple environmental factors and that the variance explained by the factor of interests can be rather low (Giordani, 2007; Jovan and McCune, 2006). We proposed here that this problem could be partially solved if we consider functional-diversity and make use of functional groups as ecological indicators. In fact, our results indicated that the effect of the environmental factors on lichens could be better explained when considering measures of functional diversity rather than measures of total diversity (Table 2). We observed that measures of total diversity grouped species with opposing responses to the environmental factors, as shown by the nitrophytic and oligotrophic functional groups under an atmospheric ammonia (NH_3) gradient (Table 2; Fig. 6 C, D in ch05). Although it has already been shown that lichen species respond in opposite directions to some environmental factors (Sparrius, 2007; Van Dobben and Ter Braak, 1998; Wolseley et al., 2006) these studies were mainly based only on the response of species, and even if the authors suggested that this response was related to species functional groups, they did not propose that this could be used in a more general framework to develop ecological indicator tools (Sparrius, 2007; Van Dobben and Ter Braak, 1998; Wolseley et al., 2006). Once again, we proposed here that functional groups can be considered accurate ecological indicators, since they respond to specific environmental factors and are robust because they are composed of multiple species, and thus independent of the composition of local communities. The functional nature of the relations between the environmental factors and lichen functional groups further supports their use as ecological indicators. One example is the use of nitrophytic species as indicators of eutrophication, since they are known to present physiological characteristics that make them more tolerant to increased nitrogen (N) availability, and even benefit from it (Gaio-Oliveira et al., 2001; Gaio-Oliveira et al., 2005). Thus, **one of the main conclusions drawn in this work was that the use of lichen functional groups as ecological indicators should be mandatory whenever the environmental factor of interest influences lichen species in different directions**, such as the case of N-pollution. However, when it has the same effect on nearly all species, total diversity measures can be used alone. That is the case of SO_2 (Hawksworth and Rose, 1970) and some metals, such as copper (Branquinho et al., 1999) because they have a detrimental effect on nearly all lichen species and functional groups.

Table 2: Comparison of the fit between environmental factors and two lichen-variables, based on total or functional diversity (nitro are nitrophytic species, oligo are oligotrophic species). The number of samples was the same between each pair. LUI is the land-use intensity (see ch4) and NH_3 the atmospheric ammonia concentration. *** P<0.001, ** P<0.01, * P<0.05.

variables	R^2
total LDV vs artificial areas (ch02.1)	0.37***
LDVoligo vs artificial areas (ch08)	0.68***
total LDV vs NH_3 (ch05)	0.33*
LDVnitro vs NH_3 (ch05)	0.90***
total number of species vs LUI (ch04)	0.21***
number of nitrophytic species vs LUI (ch04)	0.36***

If the use of functional groups intends to be a more universal tool, then we need to test the available species classification (Nimis and Martellos, 2008), which is based on expert-knowledge of habitat preferences. This test should be based on long-term experiments and done against isolated environmental factors, so that the response of species is not confounded by the influence of other factors which can have the same response pattern. In this work it has been possible for the first time to **test the classification of lichens tolerance to eutrophication against a long-time gradient of atmospheric NH_3** (Fig. 4 in ch05). With the exception of two species, that were shown to be more tolerant than expected, the classification was in good agreement with the observed position of species within the measured NH_3 gradient. These results further support the use of this classification correcting the two species, under Mediterranean climate. However, this classification should be further tested in other climates or ecoregions.

DISENTANGLE THE EFFECTS OF MULTIPLE ENVIRONMENTAL FACTORS

Since multiple environmental factors are frequently found occurring in simultaneous and since lichens respond to all present factors, there was a need to disentangle the effect of those multiple factors so that lichens could be used as reliable ecological indicators. This was achieved using several strategies.

In order to be able to focus in the environmental factors of interest, we performed a **homogenization of the effects of unwanted environmental factors at the sampling design phase** in all chapters of this thesis. Unwanted environmental factors are all those factors which have no interest for the study in question. In most cases this does not mean that the effect of these factors was removed; it means that it was held constant, so that the alterations of the lichen data could be attributed to the environmental factors of interest. One example was selecting just one tree species for sampling the epiphytic lichens in each studied case, homogenising in this way the effect of the phorophyte in lichen communities. Another example was homogenizing the effect of land-use intensity whenever land-use was not the environmental factor of interest, by sampling always sites within a narrow range of land-use intensities (ch02.1, ch02.2, ch03, ch05, ch06, ch07, ch08). This strategy was shown to work well, because data collected in a large region (Fig. 1 in ch02.1) was related to regional and local pollution sources but not to site characteristics such as forest structure of the sampling sites (Pinho et al., 2004). Homogenization of the unwanted environmental factors is a step of great importance but is rarely referred explicitly in studies using ecological indicators. Nevertheless, it should be taken into account as a central part of the sampling design in future standardizations of methodologies (Giordani et al., 2009).

As shown before, **a primary key feature for disentangle the influence of multiple factors was the use of functional diversity** which allowed the understanding of the different response of species and of functional groups to environmental factors (Fig. 3 in ch02.2; Fig. 6 in ch05). Using it we were able to map the impact of different environmental factors and also to indentify which ones were causing the effect in lichens (Fig. 3 in ch03, Fig. 5 in ch05, Fig. 6 in ch08).

Together with the use of functional diversity, a crucial and innovative step of this work was **the use of a spatial explicit analysis to increase the capacity to disentangle the impact of multiple environmental factors** (Fig. 3 in ch07; Fig. 3, 4 in ch02.1; Fig 5, 6 in ch08). Based on an analysis at different spatial scales it was possible to understand that each land-cover type had a different distance of influence in lichens and that the distance was likely related to the pollutants dispersed and to the size of the particles emitted from each land-cover type. Associating land-cover types, as a source of pollutants emitted, with its effects on biodiversity is not frequent when using ecological indicators. Moreover, when done together (Tommervik et al., 1998) it has been limited to the study of high intensity land-cover types and to a fixed scale of analysis. An example of the association between land-cover types, type of particles and its influence on lichen diversity in an analysis at multiple spatial scales was given by the longer distance of influence of ocean in LDV (Fig. 3 in ch02.1), likely associated to small size aerosols with a maximum size of 0.1 μm (Morawska et al., 1999; Piazzola and Despiau, 1997) when compared to the shorter distance found for bare-lands (Fig. 3 in ch02.1), likely associated to larger dust particles with sizes ranging up to 70 μm (McTainsh et al., 1997). Another example was the distance of impact of agriculture, which was found to be usually shorter than 600 m (Fig. 2,3 in ch02.1; Fig 3 in ch03; Fig. 3 in ch06), and was in agreement with the known distance of effect of NH_3 in biodiversity (Pitcairn et al., 2003; Pitcairn et al., 1998). Since these distances were different according to the pollutant type, it was possible to separate the areas of effect of each environmental factor making an analysis at different spatial scales. Thus, in a scenario where the influence of multiple factors is anticipated, the spatial scale of analysis should be large enough to allow encompassing several spatial scales of analysis.

In the absence of direct measures of a specific environment variable we suggest the use of neighbouring land-cover as a surrogate of the some environmental factors as it was shown in this work (Fig. 3, 4 in ch02.1; Fig. 3 in ch02.2; and Fig. 5, 6 in ch08). Thus, **the analysis of the land-cover should be used to disentangle the influence of multiple factors** on the basis that land-cover is a source of pollutants capable of influencing lichens. The permanent character of land-cover might be advantageous to measure the integrated effect of pollutants along time since the pollutant concentrations measured in the atmosphere, in general, reflect only a short-term period. It is however important to highlight that the land-use intensity of each land-cover is also an important issue for assessing the effect in lichens (Fig. 3, 4, 5 in ch04).

An additional strategy for disentangle the effects of multiple environmental factors using lichens as ecological indicators was to **make use of the capacity of lichens to accumulate pollutants**. Even making use of functional groups and of spatial analysis as depicted above may not be enough to determine which factor is causing the environmental change. The concentration of some elements/ pollutants measured in lichens may further disentangle its sources. One example was the use N concentration measured in lichens to identify the local sources of N-pollution (Fig. 5 and 7 in ch08) and to confirm that the effects observed in lichen diversity were due to N from agriculture areas and not to other factors, such as dust dispersion or to ecological responses to different light exposures which can influence lichen communities in a similar way (Loppi and Pirintsos, 2000). Using lichens as accumulators of N had the additionally advantage of finding for the first time a significant quantitative relation between N concentration in lichens and the potential amount of NH_3 emitted at a regional level (Fig. 6 in ch08).

The analysis of lichen-variables using variograms is frequently used to interpolate ecological indicators data (Figueira et al., 2009; Gay and Korre, 2006; Pesch et al., 2008), but in this work the geostatistical analysis of **the spatial characteristics of lichen variables was also used to disentangle the effect of multiple environmental factors**. For example, the spatial range of the LDVoligo was c. 10 times higher than of LDVnitro (Table 1 in ch08), which indicated that LDVoligo was likely being influenced by environmental factors working in larger areas than those influencing LDVnitro. In fact, LDVoligo could be related to factors influencing the entire region, such as NO_x (Fig. 5 in ch08) that is dispersed over several km, whereas LDVnitro could only be related to factors working at a local spatial scale such as NH_3, mostly dispersed within 1 km (Pitcairn et al., 2003). Besides range, we considered also the nugget effect of lichen-variables as a measure of the importance of the variability associated to environmental factors that work at the sampling site scale, such as tree characteristics, thus allowing the evaluation of the success of the sampling design. One example of this was the study done in a large regional area, where the nugget effect of some of the lichen-variables was near zero (Table 1 in ch02.1; Table 1 in ch05; Table 1 in ch08), ensuring that differences observed between sites were due to environmental factors working at a regional or local spatial scales, which were the environmental factors of interest.

LICHENS AS ECOLOGICAL TOOLS IN A CHANGING ENVIRONMENT

Although lichens are accepted as excellent biomonitors of many environmental changes in terrestrial ecosystems, their use as ecological indicators is only now being suggested by some authors (Will-Wolf et al., 2006). These authors justify this claim based on the observation that lichens respond to a large number of simultaneous environmental factors. In accordance to the previous works here we showed that lichens respond and integrate the effects of a large number of environmental factors. In fact, during the development of this work lichens were shown to be influenced by a large number of natural and anthropogenic environmental factors, some associated to global change drivers: pollution from industrial activities and urban areas (ch02.1, ch08), eutrophication associated to agriculture activities (ch02.2, ch03, ch04, ch05, ch06), changes in forest structure associated to management (ch04), effect of the sea (ch02.2) and climate (ch02.1, 3). Because lichens were shown to respond to a large number of simultaneously environmental factors **we could consider lichens as integrative ecological indicators of environmental changes, including the ones associated to the complex drivers of global change**. Recent studies suggested several methods to understand the effects of multiple environmental factors in lichen response, including the use of functional groups (Ellis and Coppins, 2010; Geiser and Neitlich, 2007). However these authors have used these methods to understand the distribution patterns of lichen species and communities in response to multiple environmental factors, rather than as a general framework to use lichens as integrative ecological indicators, as it was done in this work. Moreover, they did not take into account the spatial framework that is required to successfully use lichens as ecological indicators.

Lichens are known to respond to several land-use and eutrophication intensities, and some recent works tested the effects on lichens of wide eutrophication gradients under Mediterranean climate (Jovan and McCune, 2004, 2006). In this work we showed that lichens responded throughout the entire environmental gradient of agriculture land-use, including low (ch02.1, ch03, ch04), medium (ch02.1, ch08) and high intensity (ch05, ch06), by measuring both a community shift and accumulation of nitrogen, in a spatial explicit way that allowed us to disentangle the effects of eutrophication from other effects. Thus, **lichens were also shown to be wide-range ecological indicators for the effects of eutrophication**.

In fact, an important outcome of this work was that eutrophication was found to be an environmental factor present in all studied cases. Lichens were influenced by microclimate and eutrophication

(ch02.1, ch03), eutrophication and changes in forest structure (ch04), eutrophication and industrial pollution (ch02.2, ch08) and by atmospheric NH_3 (ch05, ch06). This result, observed in the studied areas and ecosystems (oak forest and woodlands) was in agreement with the suggestion that the alteration induced by humans in the N cycle is among the most important drivers associated to global changes (Rockstrom et al., 2009). In this work the dispersion (ch05) and the distance of influence (ch02.1; ch08) of eutrophication/NH_3 in lichens was found to be of short-range. However, the areas affected by it were found to be widespread in the territory (ch08), and always associated to agriculture sources (ch03, ch05, ch07 and ch08). This distribution is in accordance to the general picture given by the atmospheric NH_3 concentration in the world map (Clarisse et al., 2009): a large number of hot-spots spread across the globe located in high-intensive agriculture areas, with a patchy distribution. The latter contrast with the more spatially continuous mapping of expected climate changes expected worldwide, e.g. temperature raising (IPCC, 2007). The search of ecological indicators of climate change, another important factor associated to global change, was studied using a climate gradient driven by orography in space. These climate alterations influenced lichen communities (ch02.1 and ch03), showing that lichens can potentially be used to monitor climate change in a time-frame as already suggested by other authors (Aptroot and van Herk, 2007; Garcia et al., 2005; Giordani, 2007).

An important application of lichens as ecological indicators is to **map with high spatial resolution the areas of risk**, for example due to air pollution and microclimate alterations (Fig. 4 in ch02.1; Fig. 4 in ch03; Fig. 5 in ch05; Fig 3,5 in ch07; Fig 2,6 in ch08). We showed that lichen-variables were suitable for interpolation using geostatistical methods at a regional (Fig. 2 in ch08), local (Fig. 4 in ch03) or sub-local (Fig. 5 in ch05) spatial scales. The use of these maps may allow, for example, focusing the conservation efforts in critical areas (Fig. 3 and 5 in ch07), improving the environment management goals. Ultimately, this could allow to diminish the current lack of spatial resolution and ground truthing in current assessments of local impacts of global change factors (Hansen et al., 2010).

Lichens can be used as **ecological indicators for determining critical levels of pollutants in ecosystems**. When determining the critical levels of pollutants for a given ecosystems it is not possible to test all species, thus, within a precautionary approach, critical levels aim at protecting the more sensitive known group of species from adverse effects. Lichens are considered one of the most sensitive communities of ecosystems to air pollution, namely N-pollution (Cape et al., 2009). In this work lichens were used to determine the critical level of NH_3 in cork-oak woodlands (Fig. 4 in ch06), which was done for the first time for Mediterranean ecosystems, and was found to be very similar to values determined in other countries and climates using the same group of organisms (Wolseley et al., 2006).

Because lichens were found to be sensitive to a large number of environmental factors and to respond even to the lowest intensities of those factors, lichens could be used as **early-warning indicators for critical thresholds in ecosystems**. Critical thresholds are limits beyond which a sudden change is likely to occur in a ecosystem (Scheffer et al., 2009). The ecosystem change is not necessarily linear, thus it is important to have early-warning indicators before changes occur. Lichens, especially lichen functional groups, were shown to respond to very small environmental changes (Fig. 3 in ch02.2; Fig. 3, 4, 5 in ch04), including those related to global change such as climate and eutrophication, and could thus be used as early warning signals before damage occurs in other less sensitive components of ecosystems.

One important question left open is if **lichens could be used as *universal* ecological indicators** for the effect of environmental factors on terrestrial ecosystems. Lichens have a wide range distribution, even at the species level, are present in all land biomes, even at the more extreme ones as the polar and deserts, and are increasingly being shown to be sensitive to a large number of environmental changes. An important additional characteristic is

the possibility to define functional groups, that are not only mechanistically related to the environmental factor but are also robust measures of the community and are also independent of the local species composition (Diaz and Cabido, 2001). Using this strategy and by making use of a common sampling protocol (Asta et al., 2002) and definitions of functional-diversity (Nimis and Martellos, 2008), it was possible to show that lichen functional groups responded to the same environmental factors on a common way in two Mediterranean type ecosystems, in Portugal and Italy. The effect on lichens of NH_3 emitted from cattle was observed to be significant in the two separate countries (Table 3) but also using the merged data (Table 3). Considering that the response of lichen functional groups was significant, independently of the country, a common plot was made for the merged data-set, showing the response of lichen functional groups along an atmospheric NH_3 gradient that includes two Mediterranean-type areas located in different countries (Fig. 1).

Table 3: Spearman rank order correlations between lichen functional-diversity variables and the potential amount of emitted atmospheric NH_3, calculated for each country and considering countries together. The amount of atmospheric NH_3 was calculated from cattle census data at a civil-parish level for a 100 m circular neighbourhood around sampling sites. *** P<0.001, ** P<0.01, * P<0.05. N=64 for Italy, N=47 for Portugal, N=111 for merged dataset.

variables	ITALY	PORTUGAL	ITALY & PORTUGAL
%LDVnitro	+0.28*	+0.33*	+0.58***
%LDVmeso	Ns	Ns	-0.21*
%LDVoligo	Ns	-0.34*	-0.52***

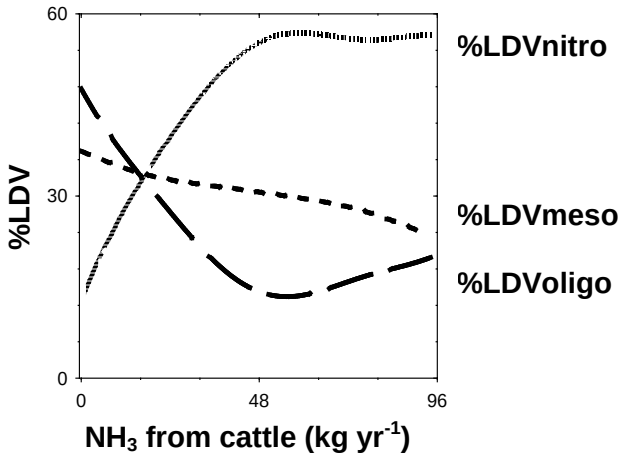

Figure 1: Plot of relative lichen functional-diversity variables against the atmospheric ammonia (NH_3) emited by cattle, calculated within a 100 m neigbourhood around sampling sites. The lines are distance-weighted least-square smooth functions (stiffness=0.35). Close symbols are from Italian area, open symbols are from the Portuguese one. N=111 for each functional-diversity variable.

CONCLUSION

This work provided a general framework for better using lichens as integrating ecological-indicators of the local effects of environmental changes in Mediterranean-type ecosystems. This was supported by the analysis of the relations between the responses of lichen communities to the environmental factors associated to drivers of global changes (climate, eutrophication and air pollution). With this work a selection of potential ecological indicators, based on total richness and functional groups was tested. The possibility of having a spatial explicit analysis allowed the disentangling of the response of ecological indicators to multiple environmental factors. Finally these indicators were applied to monitor drivers of global change in a complex Mediterranean landscapes.

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